

Selenium biochemistry and its role for human health

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Despite its very low level in humans, selenium plays an important and unique role among the (semi)metal trace essential elements because it is the only one for which incorporation into proteins is genetically encoded, as the constitutive part of the 21st amino acid, selenocysteine. Twenty-five selenoproteins have been identified so far in the human proteome. The biological functions of some of them are still unknown, whereas for others there is evidence for a role in antioxidant defence, redox state regulation and a wide variety of specific metabolic pathways. In relation to these functions, the selenoproteins emerged in recent years as possible biomarkers of several diseases such as diabetes and several forms of cancer. Comprehension of the selenium biochemical pathways under normal physiological conditions is therefore an important requisite to elucidate its preventing/therapeutic effect for human diseases. This review summarizes the most recent findings on the biochemistry of active selenium species in humans, and addresses the latest evidence on the link between selenium intake, selenoproteins functionality and beneficial health effects. Primary emphasis is given to the interpretation of biochemical mechanisms rather than epidemiological/observational data. In this context, the review includes the following sections: (1) brief introduction; (2) general nutritional aspects of selenium; (3) global view of selenium metabolic routes; (4) detailed characterization of all human selenoproteins; (5) detailed discussion of the relation between selenoproteins and a variety of human diseases.

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1. Introduction

Selenium (Se) is an essential trace element having biological functions of utmost importance for human health. Differently from the other (semi)metals, it is incorporated into proteins by a co-translational mechanism as part of the amino acid selenocysteine (SeCys), the 21st amino acid used for proteins synthesis in humans. Twenty-five Se-proteins have been identified so far in humans, whereas only few of them have been functionally characterized.^{1,2} Most Se-proteins participate in antioxidant defence and redox state regulation, particularly the families of glutathione peroxidases (GPxs) and thioredoxin reductases (TrxRs). Some Se-proteins play more specific essential roles, such as iodothyronine deiodinases (DIOs) which are involved in thyroid hormones metabolism, GPx4 which is essential for spermatogenesis, and selenophosphate synthetases 2 (SPS2) participating in Se-proteins biosynthesis. Other Se-proteins may also be involved in important biological processes, but their exact mechanism of action is still not fully understood.

Despite the scarce knowledge of the precise biochemical functions, a very large number of studies have been carried out in the last two decades showing that insufficient Se levels, and particularly Se-proteins, are associated with several human diseases including cancer, diabetes, cardiovascular and immune system disorders.³ In most cases, the link lies in the contrast to the oxidative stress that may be both causing or caused by the disease. In this context, it is important to decipher whether an adequate Se status may contrast the risk factors for health disorders, or Se supplementation may improve the therapy when Se metabolism is altered. Additional attention was recently driven by the finding that Se-proteins' genes polymorphism is associated to cancers and other diseases.⁴ Cancer research is one of the most promising lines, in which Se has been used experimentally as a key component of newly designed anti-cancer drugs.⁵

Even if still incomplete, the broad literature supporting the importance of selenium for human health has yielded great interest in Se supplementation. Despite many studies that have suggested a beneficial effect from Se supplementation to general health protection, most of them have remarked that it is limited to the initially inadequate Se status.⁶ Conversely, care should be taken when using supplements because excessive Se intake leads to toxic effects, and recent studies have shown that even sub-toxic doses may be negatively impacting, for example by increasing the risk of type 2 diabetes.⁷

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Deepening the knowledge of Se biochemistry in humans, as well as its integration at clinical and epidemiological levels, is important to extricate the relationship between Se status and the efficiency of biological systems. This is particularly challenging when taking into account that such a link is expected to be species-specific and system-specific, so that a given Se status may be optimal for some functions but not for others.

2. Nutritional aspects

The comprehensive characterization of Se nutritional features is beyond the aim of this work. Nevertheless, some key aspects

are important in terms of their bio-medical applications, because any study aiming at the elucidation of Se biochemistry must be contextualized within the nutritional status of the subject. The main challenges in Se nutrition are: (i) the accurate assessment of the Se dose taken in by the subjects under investigation; (ii) establishing the appropriate markers for the assessment of Se status; (iii) identification of the confounding factors allowing the conversion of Se intake into Se status. Such challenges outline a complex scenario, which often clashes with the need for simplicity of communication with the general public and administrations.

2.1. Selenium in food

The main route for Se intake is *via* the diet, whereas the contribution from water and air is negligible.⁶ The total amount of Se in the diet varies widely depending on the food type and composition. The major contributors to Se intake is typically provided by bread and cereals, meat, fish, eggs, and milk/dairy products. An estimation of Se levels in different types of food was recently reviewed.^{6,8}

The level of Se in crops is related to that in the soil; the bioavailability is regulated by physicochemical conditions of the soils such as the pH, redox conditions, salinity, organic matter, *etc.*⁹ Crops are an important source of Se taking into account their consumption on a global scale. Nevertheless, Se in crops is generally of low abundance because such plants do not require Se for growth, and hence do not accumulate it under normal physiological conditions. A level ranging between 10–550 $\mu\text{g kg}^{-1}$ of Se (fresh wt) was reported in cereals,¹⁰ whereas Se in bread is generally found at a level of $\sim 60\text{--}160 \mu\text{g kg}^{-1}$.⁹ Other vegetables such as those of the *Allium* family, including garlic and onion, can accumulate significant amounts of Se,



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reaching concentrations of Se up to 68 and 96 $\mu\text{g g}^{-1}$, respectively.¹¹ High amounts of Se can be accumulated also by mushrooms and broccoli. The richest natural source of Se are Brazil nuts, which have a mean concentration of Se up to 83 $\mu\text{g g}^{-1}$.¹²

In animal products, the level of Se reflects the levels used in cattle feed. In meat, eggs, and particularly fish, which are protein-rich, the Se content is relatively high, in the range of $\sim 49\text{--}739 \mu\text{g kg}^{-1}$ (fresh wt).⁹ Additionally, specific organs, such as liver and kidney, may contain a particularly high concentration of Se, up to 1500 $\mu\text{g kg}^{-1}$.⁹

A number of approaches can be used to estimate the dietary intake of Se, ranging from direct analysis of composite food types, to indirect calculation using dietary or market basket surveys and food composition tables.¹³ Notably, since the total concentration of Se in food strongly reflects the soil conditions, the dietary intake varies widely with geographical localization.¹⁴

An additional crucial aspect is that food types provide Se in distinct combinations of chemical forms which in turn entail a different bioavailability of the element.¹⁵ The main Se-species in vegetables are selenomethionine (SeMet) and selenate/selenite (SeO_4^{2-} , SeO_3^{2-}); minor species are SeCys, Se-methyl-selenocysteine (SeMCys) and γ -glutamyl-Se-methyl-selenocysteine (GGSeMCys).¹⁵ Selenate/ite, SeMet and SeCys are the main species in animal products, with widely variable proportions depending on the animals' diet. The distribution of Se species in food varies considerably depending on the plant/animal species, the environment and the growth conditions (natural or supplemented).¹⁵ As is discussed in the following sections, each species is characterized by specific absorption/assimilation routes and efficiency, and hence the speciation analysis of Se in food is a key requisite to accurately depict the relationship between intake and health status, especially where supplementation is concerned.

2.2. Assessment of Se nutritional status

Selenium is an essential element presenting a very narrow range between deficient, essential and toxic doses.¹⁰ The assessment of optimum Se dietary requirements is still a matter of debate. Until few years ago, most of the studies focusing on Se status assessment investigated only the total level of the element in tissues or body fluids. Plasma or serum Se concentration was generally considered a useful biomarker of both Se status and dietary intake in the short-term, whereas erythrocyte Se reflects better the long-term status.¹⁶ Other tissues were also used to measure long-term Se status, including hair and toenails. Daily urinary excretion closely associates with plasma Se level and dietary intake accounting for a stable value of 50–60% of the total amount excreted, and thus was also used as short-term intake measure.

Recently, it has been pointed out that total Se concentration is not representative of the real functional activity of Se, because the element is incorporated in a large variety of proteins with different biological functions.¹⁶ The distribution of Se among Se-proteins is strongly dependent on a precise hierarchy in its incorporation, the average dietary intake, the speciation of Se in food, the health state, age, lifestyle (smoking and exercise), and also by genetic polymorphism of Se-proteins.¹⁶ Thus, the measure

of individual Se-proteins activity is expected to constitute a more accurate biomarker for the functional status of Se. In this respect, the most frequently used parameter nowadays for the assessment of Se status is the activity of the plasma Se-protein glutathione peroxidase (GPx3), compared to its maximum. The activity of GPx3, as well as erythrocyte GPx1, are well correlated with the total level of Se in blood until a maximum is reached at $\sim 100 \text{ ng mL}^{-1}$, corresponding to approximately 70 μg per day intake.¹⁶ Plasma selenoprotein P (SeP) has also been proposed as a biomarker, reaching maximum expression at a slightly higher blood Se level, namely $\sim 124 \text{ ng mL}^{-1}$ corresponding to $\sim 105 \mu\text{g}$ per day intake.¹⁷ The concentration of SeP reflects mostly the short-term status of Se in the organism because it has a half-life in plasma of few hours (3–4 h in rat plasma).¹⁸ This makes it a better marker than GPx3 for the assessment of the Se nutritional status. Nevertheless, once the basal Se requirement is reached, additional increase of the element does not lead to an increase in GPx3 or SeP concentration, therefore such Se biomarkers have limited validity.¹⁹

Plasma/serum selenoproteins, namely SeP and GPx3 are the most commonly used markers for the assessment of Se status also because they can be determined with a scarcely invasive procedure compared to tissue Se-proteins, which requires a biopsy. It is worth highlighting that the choice of Se-protein to be used as a biomarker must consider its specific biological function, which therefore provides partial information in terms of Se bioactivity. In fact, the most efficient biomarker is expected to be not a single protein, but rather a set of combined parameters, being applied to a specific problem associated with suboptimal Se status; an example could be the expression of Se-proteins mRNA circulating leukocyte.²⁰ The integration of these biomarkers with the comprehensive analysis of health parameters, endocrine and immunological status, Se-proteins polymorphism and other variables is considered nowadays as the most promising approach.²⁰

An alternative approach for Se status assessment is the comparison of dietary Se intake with the specific end-point of a disease. The basal Se requirement should be the intake allowing the prevention of pathologically and clinically relevant signs of dietary inadequacy. This was the approach used in case of Keshan disease, an endemic disorder found in Se-deficient areas.²¹ More recently, the evolution towards recognition of an 'optimal nutrition' has moved interest to the possible health effects of Se in larger than minimum intakes, considering the alternative end-points of the promotion of growth, maintenance of good health and reduction of other diseases not caused by nutritional deficiencies.⁶ Nevertheless, the causal association between Se-proteins and specific diseases is still far from being clarified, and its inclusion in the estimation of the recommended Se intake dose appears to be premature.

2.3. Recommended daily intake levels

Several institutions have proposed reference values for the daily recommended dietary allowance (RDA) for Se, taking into account the most reliable epidemiological studies. An intake of $\sim 20 \mu\text{g}$ per day for adults is generally accepted as the minimum needed to prevent Keshan disease onset.²¹ Considering the more general prevention of pathologically and clinically relevant signs of dietary inadequacy, the World Health Organization (WHO)

corrected this basal requirement to an average of 19 μg per day, corresponding to 21 μg per day for men and 16 μg per day for women, taking into account body weight.²² Other criteria were based on GPx3 activity maximization for the calculation of the recommended level; in this respect, the RDAs provided by the U.S. Department of Agriculture are mostly adopted as a general reference.²³ Most of the recommended daily intake levels range between 50 and 60 μg per day, with small variations between genders and for particular categories (*i.e.* pregnancy) or age groups.

Providing upper limits for Se intake is difficult because there are limited data regarding the Se toxicity for humans. So far, acute toxic symptoms have been associated with extremely high Se intakes ranging between 3200 and 6700 μg per day, but symptoms such as fingernail changes have also been reported for Se intakes of 1260 μg per day.²⁴ Interestingly, some studies did not report any observed adverse effect level (NOAEL) for an intake <800 μg per day for adults, while others reported selenosis in case of Se intakes ≥ 850 μg per day.²⁵ In this context, the US Environmental Protection Agency has defined an intake level of 1262 μg per day as the reference at which clinical selenosis appears.

More difficult to estimate is the association between high dietary Se intake and diseases which are not directly caused by the element, such as cancer. For example, the Nutritional Prevention of Cancer Trial found an increased risk of squamous cell carcinoma and total non-melanoma skin cancer in individuals with a high basal risk supplemented with 200 μg per day of Se,²⁶ but there are still doubts about how this would relate to risk for the general population. From a preventive perspective, the National Health and Medical Research Council (NHMRC) choose to apply to this latter estimate as a factor of 2 to protect sensitive individuals from gaps in the data and incomplete knowledge. The upper limit was therefore set at 400 μg per day for all adults, as there are no data to suggest increased susceptibility during pregnancy and lactation.²⁷

2.4. Se supplementation

Because of the low abundance of soil Se in some areas around the globe, a wide variety of Se-enriched materials have been produced in order to supply the population with Se to meet the levels adequate to the RDAs. Several strategies have been followed to obtain such products. The use of fertilizers enriched in sodium selenite is one of the most commonly used techniques to obtain vegetables with high Se concentration, a strategy which has been used in Finland since 1984.²⁸ The use of fertilizers supplemented with Se is very effective and is easily controlled to favour accumulation by plants, and plant growth itself is generally enhanced by this element; however, plant growth may be reduced by feedback mechanisms when the Se concentration is too high.²⁸ Plants having the ability to accumulate high concentrations of Se such as broccoli,²⁹ garlic,³⁰ green onions,³¹ green tea³² and mushrooms,³³ are particularly adapt at obtaining natural dietary supplements following a fertilization strategy. As a consequence of Se fertilization, an increase of Se levels in milk, meat, eggs and the whole food chain has been observed.²⁸ The total Se intake in Japan,

Australia, Finland, and the USA, as well as Keshan areas in China has been significantly increased in the last decades by the diffusion of Se-enriched fertilizers.⁹ However, Se speciation in these fertilizing products plays a differential role which has not yet been clearly elucidated.

The use of Se-enriched fertilizers has been effective, but Se is partially lost during harvesting and manipulation prior to uptake by the plants.¹⁰ An alternative is supplementation of animal feed to become enriched in Se. This strategy includes: (i) direct application of Se to pastures to increase Se uptake by plants for animal feed; (ii) supply of sodium selenite or selenate incorporated into salt blocks or licks; (iii) direct administration of Se to animals by yeast-based supplements or by drenching the feed with Se salt solutions such as sodium selenite; and (iv) use of Se pellets that is slowly released into the gut of the animal.⁹ Recently, a technological process to produce Se-enriched eggs, meat and milk has been developed and successfully introduced in various countries worldwide.³⁴ Nevertheless, detailed investigations into the possible interactions with other nutrients in Se-enriched food are still missing.

Direct intake of Se supplements by humans has also received considerable attention in recent years. Two types of multimicronutrients are commonly used such as: (i) multi-vitamins and multi-mineral preparations containing inorganic Se, other trace elements and vitamins, and (ii) supplements based on *Saccharomyces cerevisiae* yeast (Baker's yeast).⁹ The selenized yeast is particularly attractive due to its low cost, facility to grow under different conditions, and its ability to assimilate up to 3000 $\mu\text{g g}^{-1}$ of Se starting from sodium selenite added to the growth medium. Se-enriched yeast is currently the primary Se dietary supplement, where Se is present mostly as SeMet.³⁵ Several minor organic species have been reported, such as methylselenol, SeCys, seleno-homocysteine, selenoadenosine and others at trace levels.³⁵

3. Metabolic routes of Se in humans

3.1. Absorption and metabolism

The global metabolism of Se in mammals is schematically represented in Fig. 1. The main pathway for the assimilation of Se intake was first proposed by Ip³⁶ and consists of the reduction of the different species to hydrogen selenide (HSe^-). This species plays the role of a central gateway for both utilization and excretion of Se. Selenium excess detoxification occurs through a mechanism of sequential methylation into dimethylselenide (DMSe), excreted into the breath, and selenosugars and trimethylselenide (TMSe), which are excreted into the urine.

The absorption of Se-species occurs mainly in the lower part of the small intestine by different routes and mechanisms, in many cases shared with their sulphur analogues. Almost all forms of Se, inorganic as well as organic, are readily absorbed with an overall efficiency close to be complete (70–90%) under normal physiological and intake conditions.³⁷ Selenite is an exception because its direct absorption does not exceed 60%. However, in the presence of reduced glutathione (GSH), as occurs in the gastrointestinal fluid, the absorption of selenite is increased

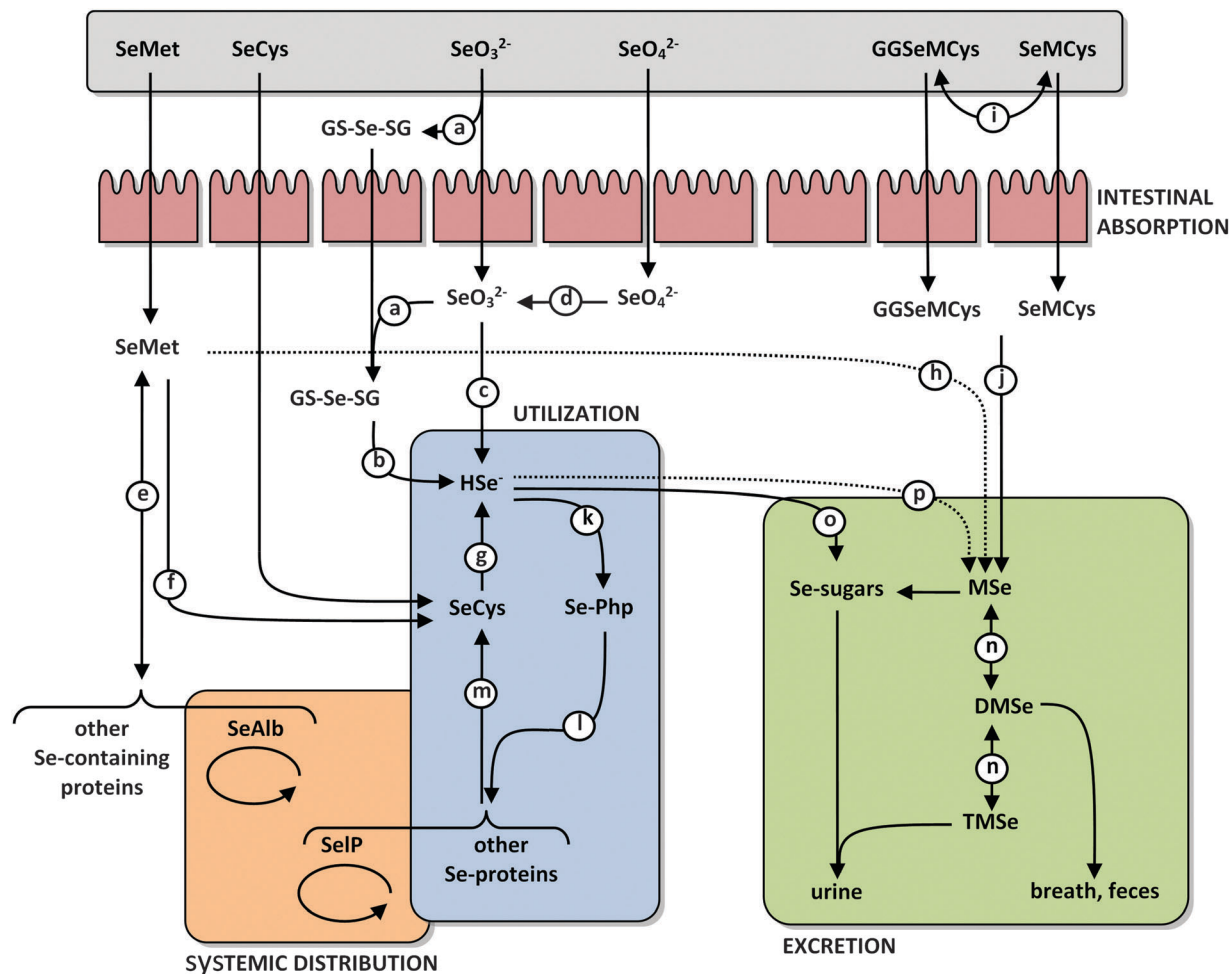
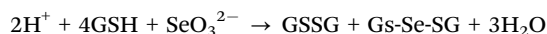
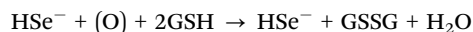
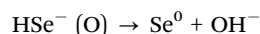
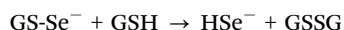
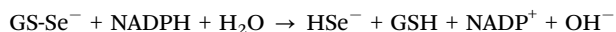
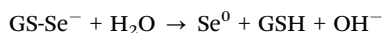
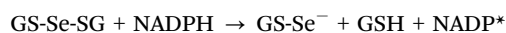


Fig. 1 Global view of Se metabolism in mammals.

up to quantitative proportions.³⁸ In these conditions, selenite reacts non-enzymatically with thiol groups of GSH to form selenodiglutathione (GS-Se-SG, Fig. 1 path a), as follows:³⁹



GS-Se-SG is subsequently decomposed by glutathione reductase into selenide following the steps (Fig. 1 path b):



The GS-Se-SG should remain stable in the stomach due to the low pH conditions, but it is expected to become unstable

and reactive in the intestine.⁴⁰ The transport proteins involved in the direct or indirect absorption of selenite are not yet known. The fraction of selenite which is directly absorbed undergoes the same reduction in red blood cells (RBCs), so that the overall pool of the species is converted into selenide.⁴¹ Alternatively, selenite can be a substrate for the thioredoxin system (thioredoxin, NADH and thioredoxin reductase, itself a Se-protein) and directly reduced to selenide (Fig. 1 path c) following a reaction path similar to that reported above for glutathione reductase.³⁹ The diglutathione (GSSG) is not a substrate for thioredoxin reductase and is a poor disulfide substrate for reduced thioredoxin. Nevertheless, the insertion of a Se atom makes this compound a highly reactive substrate for the thioredoxin system, capable of redox cycling in the presence of oxygen.

Selenate is absorbed paracellularly, with elevated efficiency, *via* a passive diffusional process.³⁸ After absorption, it is reduced to selenite (Fig. 1 path d), as in sulfate reduction, by ATP sulfurylase *via* the still unidentified Se-isologue of 3-phosphoadenosine 5-phosphosulfate (Se-PAPS).

The Se-amino acids SeMet and SeCys are absorbed through transcellular pathways mediated by transporters which are

basically shared with their sulphur-containing analogues.⁴² SeMet is absorbed through a Na⁺-dependent process, but the identity and affinity of the transport proteins is still to be established.

SeMet can also be incorporated non-specifically into proteins such as serum albumin and haemoglobin, by randomly replacing the (sulphur) methionine (Fig. 1 path e).⁴³ Alternatively, it can be transformed into SeCys (Fig. 1 path f) and then into selenide (Fig. 1 path g) via the *trans*-selenation pathway, analogous to the *trans*-sulfuration pathway,⁴⁴ schematized in Fig. 2. The SeMet released through protein catabolic processes enters the *trans*-selenation pathway in the same way. Excess of SeMet has been also proposed to undergo direct methylation by γ -lyase (Fig. 1 path h).⁴⁵

The absorption of SeMCys may share with SeMet part of the transport mechanism, but some distinctions are still not clearly understood.⁴² The Se-dipeptide GGSeMCys is assumed to play the role of a carrier of SeMCys. After ingestion as a dietary constituent, the bulk (not necessarily the entire amount) of GGSeMCys is hydrolyzed by γ -glutamyl transpeptidase in the gastrointestinal tract (Fig. 1 path i), releasing SeMCys for absorption and systemic delivery to the other tissues.⁴⁶ GGSeMCys is quantitatively absorbed from the gastrointestinal tract like

SeMCys. SeMCys and GGSeMCys are directly methylated by β -lyase to MSe (Fig. 1 path j) so that urinary excretion is the major route for eliminating the excess of Se from these species.⁴⁵

3.2. Utilization

The utilization of selenium requires the generation of Se-donor selenophosphate (SePhp) from selenide and ATP (Fig. 1 path k) that is mediated by selenophosphate synthetase 2 (SPS2). Different from all the other amino acids that are synthesized before being aminoacylated onto their tRNAs, SeCys is directly synthesized on its tRNA, designated tRNA^{[Ser]^{Sec}}, by the mechanism represented in Fig. 3.⁴⁷ The tRNA^{[Ser]^{Sec}} is initially aminoacylated with serine by seryl-tRNA synthetase (SerRS). The hydroxyl moiety of Ser is then replaced by a phosphate group to form O-phosphoseryl-tRNA^{[Ser]^{Sec}} by a specific kinase (PSTK). Finally, SeCys synthase (SeCysS) exchanges the phosphate group with activated SePhp to form selenocysteyl-tRNA^{[Ser]^{Sec}}. The tRNA^{[Ser]^{Sec}} reads the UGA codon and is used for the integration of SeCys into the amino acidic sequence to form Se-proteins (Fig. 1 path l).⁴⁷ Thus, SeCys is recognised as the 21st amino acid because its synthesis is genetically encoded in the ribosome-mediated system. Interestingly, in mice Cys can replace SeCys in Se-proteins such as thioredoxin reductases in

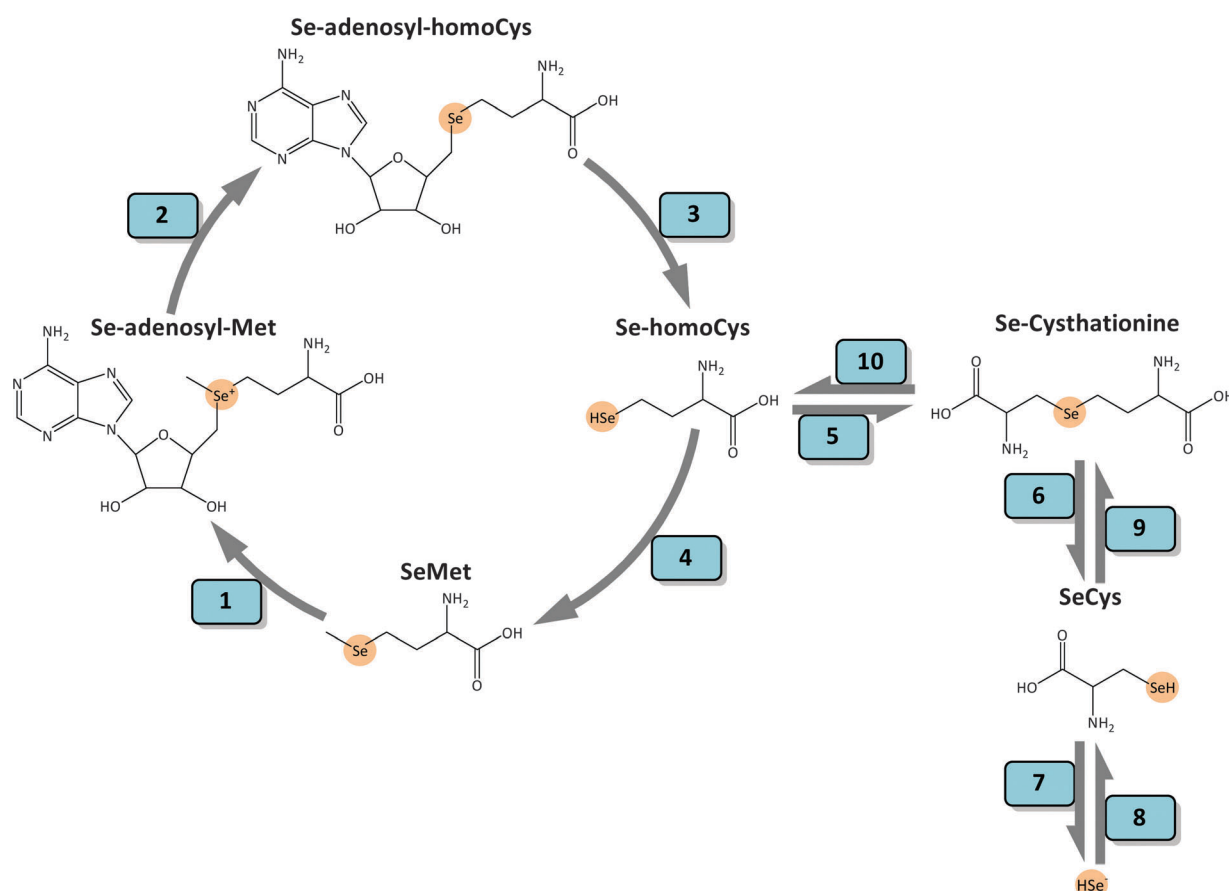


Fig. 2 Se-compounds generated within the *trans*-selenation pathway. The involved enzymes are: 1, SAM synthetase; 2, methyl transferase; 3, SAH hydrolase; 4, methionine synthase; 5, cystathionine β -synthase; 6, cystathionine γ -lyase; 7, cysteine lyase; 8, cysteine synthase; 9, cystathionine γ -synthase; 10, β -cystathionase.

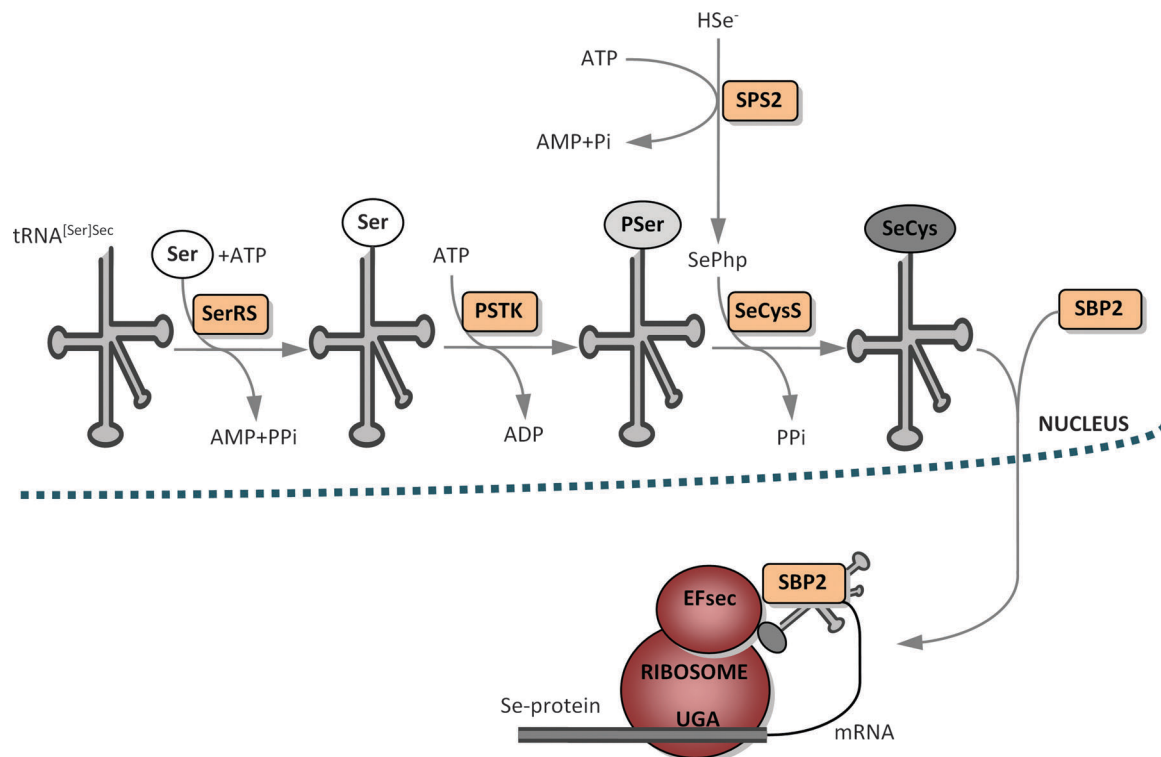


Fig. 3 Scheme of the Se-proteins biosynthesis pathway.

proportions that depend on the Se status.⁴⁷ The catabolism of Se-proteins releases SeCys (Fig. 1 path m) which is cyclically reconverted into selenide.

3.3. Systemic distribution

The Se-species absorbed into the gastro-intestinal tract are firstly transported into the liver: SeMet is usually transported in the form of Se-albumin (SeAlb)⁴⁴ while selenate and the other organic species may be transported intact or through mechanisms which are still not elucidated. The liver is the foremost organ in Se metabolism, since it synthesizes most of the Se-proteins and regulates the excretion of Se metabolites.⁴⁸ The SeIP produced into the liver is released into the bloodstream and is responsible for the distribution of Se to the other organs, where other Se-proteins can be synthesized. The local uptake of Se from plasma has been shown to occur by endocytosis mediated by receptors of the apolipoprotein family such as apoER2 in testis and brain,⁴⁹ and megalin (Lrp2) in kidney.⁵⁰ Thus, the liver regulates the whole-body Se distribution by sorting the metabolically available Se between the two pathways of Se-proteins synthesis and the excretory metabolite synthesis.⁵¹ Such regulation might be passive, so that the fraction of Se that cannot be utilized for Se-proteins synthesis enters the excretory pathway. Active regulation of the excretory metabolites has been also hypothesized,⁵¹ but not yet investigated.

3.4. Excretion

The excretion of Se in humans follows two possible routes, leading in both cases to methylated products. The proportion

among the main metabolites depends on the source species and the Se status. Under supplemented or toxic Se status, TMSe is well established as the main metabolite.⁵² Its production starts from Se sources which are already mono-methylated species, such as SeMCys and selenobetaine (SeBet), and is subsequently transformed following a stepwise methylation pathway mediated by methyltransferases (Fig. 1 path n).⁵³ The formation of the intermediate species, DMSe, excreted through the breath, seems to be kinetically favoured with respect to TMSe.

Under low-toxic Se status the metabolism of Se follows another route, where selenide is converted into an intermediate selenosugar-GS conjugated (GS-Se-*N*-acetyl-galactosamine, GS-SeGal) and then into SeMethyl-*N*-acetyl-galactosamine (MSeGalNAc), excreted into the urine (Fig. 1 path o).⁴¹ Minor selenosugars have been also detected in urine, including SeMethyl-*N*-acetyl-glucosamine (MSeGluNAc)⁵⁴ and the deacetylated analogue of SeGalNAc, SeMethyl-*N*-amino-galactosamine (MSeGalNH₂).⁵⁵ It has been also hypothesized that in case of Se excess, HSe⁻ can be metabolized entering into the stepwise methylation pathway (Fig. 1 path p).⁵³

4. Selenoproteins

Selenium is the key component of the active site of several Se-proteins having essential biological functions. Twenty-five Se-proteins have been identified in the human proteome² and 24 in rat and mouse proteome.⁵⁶ The main characteristics of human Se-proteins are summarized in Table 1. Most Se-proteins

Table 1 Human Se-proteins

Protein	Tissue distribution	Subcellular location	Mw (kDa)
Glutathione peroxidases (GPxs) family			
GPx1 (cGPx)	Ubiquitous, highly expressed in erythrocytes, liver, kidney, lung	Cytoplasm	87 (tetramer)
GPx2 (GPx-GI)	Liver, epithelium of the gastrointestinal tract	Cytoplasm	93 (tetramer)
GPx3 (pGPx)	Plasma	Secreted	93 (tetramer)
GPx4 (PHGPx)	Testes	Cytoplasm, mitochondria, nucleus	22
GPx6	Olfactory epithelium, embryos	Secreted	23
Thioredoxin reductases (TrxRs) family			
TrxR1 (TxnRd1)	Ubiquitous	Cytoplasm, nucleus	60–108 (dimer, 4 isoforms)
TrxR2 (TxnRd2)	Ubiquitous; highly expressed in the prostate, ovary, liver, testes, uterus, colon, small intestine	Mitochondria	60–106 (dimer, 4 isoforms)
TrxR3 (TxnRd2, TGR)	Testes	Cytoplasm, nucleus, ER, microsome	75
Iodothyronine deiodinases (DIOs) family			
DIO1	Liver, kidney, thyroid, pituitary gland, ovary	ER and plasma, membrane	4–29 (9 isoforms)
DIO2	Thyroid, heart, brain, spinal cord, skeletal muscle, placenta, kidney, pancreas	ER membrane	30, 34 (2 isoforms)
DIO3	Placenta, fetal tissues, skin	Cell and endosome membrane	31
Se-proteins 15 and M family			
SelM	Mainly brain; kidney, lung and other tissues	Perinuclear region, ER lumen, Golgi	14
Sep15 (15 kDa Se-protein)	High levels in prostate and thyroid gland; lung, brain, kidney, H9 T cells	ER lumen	15, 13 (2 isoforms)
Se-protein S and K family			
SelS (VIMP)	Plasma, various tissues	ER membrane	21
SelK	Various tissues; abundant in heart	ER membrane	10
Rdx proteins family			
SelW (SEPW1)	Various tissues, abundant in muscles	Cytoplasm	9
SelH	Various tissues, mainly expressed in embryonic and tumor cells	Nucleus	13
SelT	Ubiquitous	ER, Golgi	20
SelV	Testes	Unknown	17
Other Se-proteins			
SelP (SEPP1)	Expressed in the liver, heart and brain, secreted into the plasma. Also found in the kidney.	Secreted	45–57 (3 isoforms, glycosylated)
SPS2	Liver	Cytoplasm	47
SelR (MrsB1, SelX)	Heart, liver, muscle, kidney	Cytoplasm, nucleus	5–14 (2 isoforms)
SelN	Ubiquitous; abundant in skeletal muscle, brain, lung, placenta	ER membrane	61–62 (2 isoforms, glycosylated)
SelI (hEPTI)	Various tissues; abundant in brain	ER membrane	45
SelO	Various tissues	Unknown	73

exhibit antioxidant activities, but other specific processes have been linked with Se-proteins, including biosynthesis of deoxyribonucleoside triphosphates (dNTPs) for DNA, reduction of oxidized proteins and membranes, redox regulation of transcription factors, regulation of apoptosis, immunomodulation, regulation of thyroid hormones, selenium transport and storage, protein folding and degradation of misfolded proteins in the endoplasmic reticulum (ER). It is worth noting that for many Se-proteins the biochemical role is still partially unknown.¹¹

Except for SelP, all Se-proteins contain one SeCys residue solely which plays a central role in defining their biochemical activity. Based on the location of the SeCys residue, the Se-proteins can be divided into two groups.⁵⁷ One group comprises of thioredoxin reductases, SelK, SelS, SelR, SelO, and SelI, where SeCys is located in the C-terminal region. The second group includes all the other Se-proteins, having the SeCys residue in the N-terminal region. All Se-proteins are sensitive to the overall intake of Se according to a hierarchy, which depends on the

specific tissue and the biological functions under examination.⁵⁸ This section presents the main and most recently discovered characteristics of Se-proteins, with particular emphasis on their biochemistry.

4.1. Glutathione peroxidases

Glutathione peroxidases (GPxs) are a family of enzymes with antioxidant functions. The GPxs family comprises of eight isoforms, but only five members have a SeCys residue and can catalyze the reduction of hydrogen peroxide (H_2O_2) and lipid hydroperoxides using GSH as a reducing cofactor.⁵⁹ This group comprises of the ubiquitous cytosolic GPx (cGPx, GPx1), gastrointestinal GPx (GI-GPx, GPx2), plasma GPx (pGPx, GPx3), phospholipid hydroperoxide GPx (PHGPx, GPx4) and the olfactory epithelium GPx (GPx6). The SeCys residue is oxidized by the peroxide with the formation of selenenic acid, which is then reduced back to the selenolate by thiols according to the scheme in Fig. 4.⁶⁰ The selenolic group of GPx active site is

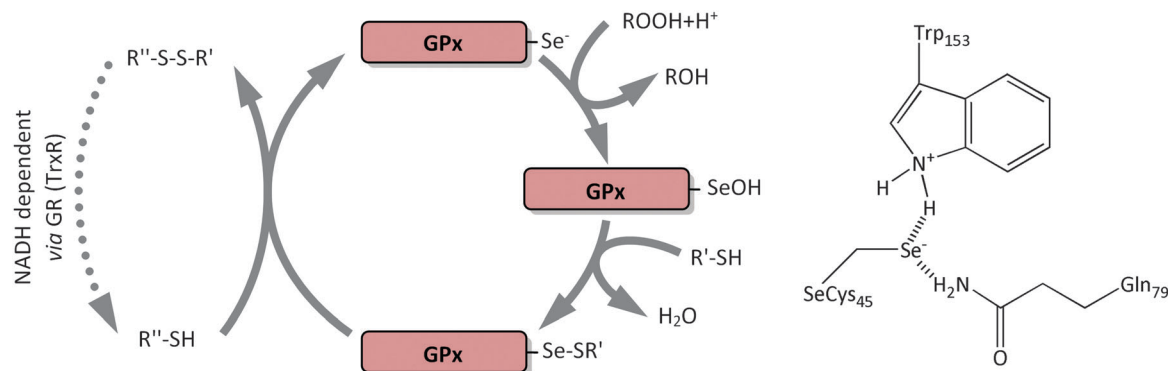


Fig. 4 Scheme of the catalytic activity of GPxs (left) and active site of the protein showing the catalytic triad (right).

included into a catalytic triad of SeCys-Trp-Gln residues where it is both stabilized and activated by hydrogen bonding.⁶⁰ A specific ranking characterizes GPxs in terms of Se incorporation, which is supposed to be representative of their relative biological importance, namely GPx2 > GPx4 >> GPx3 = GPx1.⁶¹

GPx1 is a ubiquitous homotetrameric protein localized in the cytosol and mitochondria. This enzyme utilizes exclusively GSH as a substrate for the reduction of H₂O₂ and a limited number of organic hydroperoxides including cumene hydroperoxide and *tert*-butyl hydroperoxide.⁵⁹ The reactions mediated by GPx1 mean this enzyme is implicated in the cellular processes modulated by hydroperoxides, including cytokine signalling and apoptosis. Among its family members, GPx1 is one of the most highly sensitive to changes in both Se status and oxidative stress conditions,⁶² but it appears that global protein synthesis is reduced under conditions of stress as a means of reserving cellular resources, and that GPx1 recovers rapidly compared to the other Se-proteins.²

GPx2 is a secreted homotetrameric enzyme mainly expressed in the gastrointestinal system mucosa, including the squamous epithelium of the esophagus; and in humans, it is also detectable in the liver. Its expression in the intestine is not uniform, but it is higher in the crypt grounds and decreases gradually toward the luminal surface, suggesting a role in cell proliferation.⁶³ The function of GPx2 is mainly to protect intestinal epithelium from oxidative stress and to guarantee mucosal homeostasis. GPx2 exhibits substrate specificity similar to that of GPx1, which includes H₂O₂, *tert*-butyl hydroperoxide, cumene hydroperoxide, and linoleic acid hydroperoxide, but not phosphatidylcholine hydroperoxide.⁶⁴ The expression of GPx2 is much more resistant than GPx1 or GPx3 to dietary Se deficiency.⁶¹ GPx2 location and resistance suggest that this Se-protein may serve as a first line of defence in exposure to oxidative stress induced by ingested prooxidants or gut microbiota.

GPx3 is the only extracellular enzyme of the GPxs family. It is a glycosylated homotetrameric protein produced into the cells of the proximal tubular epithelium and in the parietal cells of Bowman's capsule of the kidney.⁶⁵ Part of GPx3 is then secreted into the plasma, where it constitutes approximately 15–20% of

the total Se, but a major fraction remains bound to the basement membranes in kidneys.⁶⁵ Such membrane binding-ability has been demonstrated also in the gastrointestinal tract, the lung, and the male reproductive system.⁶⁶ Both GPx3 protein and mRNA have been also detected in several tissues, particularly the heart and thyroid gland, where this enzyme may play a role in a local source of extracellular antioxidant capacity.⁵⁸ Unlike GPx1, GPx3 presents a more restricted hydroperoxide substrate specificity. Although it can reduce H₂O₂ and the same organic hydroperoxides, its activity is ~10 fold lower than the activity of GPx1. Considering that GSH is a poor reducing substrate for GPx3 and the low concentration of reduced thiol groups in human plasma, it has been proposed that binding of GPx3 to the basement membrane exposes the enzyme to higher levels of secreted GSH, thus increasing the activity of GPx3 at the basal extracellular aspect of epithelial cells.⁶⁶

GPx4 is a monomeric intracellular enzyme presenting three isoforms: cytosolic, mitochondrial, and nuclear. The expression and activity of this protein has been documented in many tissues, particularly of endocrine organs and in the mitochondria in the midpiece of spermatozoa, and is hormone-regulated.⁶⁷ Unlike the other GPxs, it can directly use phospholipid hydroperoxide as substrate, and reduces H₂O₂, cholesterol, cholesteryl ester- and thymine-hydroperoxides, by using electrons from protein thiols as well as from GSH.⁶⁸ GPx4 plays essential role of antioxidant defence during cellular differentiation in embryonic development and in spermatogenesis and is involved in the condensation of chromatin during spermatogenesis.⁶⁹ It is also a structural protein in spermatozoa: the nuclear isoform contributes to posttesticular chromatin condensation *via* disulfide bridging in thiol-containing protamines, while the mitochondrial isoform participates to the structural organization of mitochondria in the sperm midpiece.⁷⁰ A recent study has shown that GPx4 plays an important protective role for photoreceptor cells against oxidative stress.⁷¹

GPx6 is a close homolog of plasma GPx3. Compared to other GPx proteins, GPx6 was identified rather late because its mouse and rat orthologs had Cys in place of SeCys. This enzyme is only expressed in embryos and olfactory epithelium,¹ and its specific function remains unknown.

4.2. Thioredoxin reductases

The thioredoxin reductases (TrxRs) are homodimeric enzymes belonging to the flavoprotein family of pyridine nucleotide-disulphide oxidoreductases, which includes lipoamide hydrogenase, glutathione reductase and mercuric ion reductases. Three isoforms were identified in mammals: cytosolic (TrxR1), mitochondrial (TrxR2), and thioredoxin glutathione reductase (TGR, TrxR3).⁷²

As is the case in other enzymes of the flavoproteins family, each monomer of TrxRs includes a FAD prosthetic group, a NADPH binding site and an active site containing a redox-active disulphide. The two subunits participate in the activity of the enzyme in a coordinated way.⁷² Electrons are transferred from NADPH *via* FAD to the active site disulphide of TrxR, which then reduces the substrate as represented in Fig. 5. TrxRs specifically reduces oxidized thioredoxins (Trxs), a group of small (10–12 kDa) ubiquitous redoxactive peptides that supply reducing equivalents to the disulphide bonds in enzymes such as ribonucleotide reductase, thioredoxin peroxidase, and some transcription factors, resulting in their increased binding to DNA and altered gene transcription.⁷² Mammalian Trxs have also been shown to act as cell growth factors and to inhibit apoptosis. Since TrxRs are the only class of enzymes known to reduce oxidized Trx, it is possible that alterations in TrxR activity may regulate some of the activities of Trxs.

In addition to Trxs, many other endogenous substrates have been identified for TrxRs, including lipoic acid, lipid hydroperoxides, the cytotoxic peptide NK-lysin, dehydroascorbic acid, the ascorbyl free radical, Ca-binding proteins, glutaredoxin 2, and the tumour-suppressor protein p53.⁷³ However, the physiological role that TrxRs play in the reduction of most of these substrates is still unknown. Some of the most likely functions for TrxRs are summarized in Fig. 5. The ability of TrxR to reduce the ascorbyl free radical suggests that TrxR may play an additional action through the recycling of ascorbate.⁷⁴ Humans lack the ability to synthesize ascorbic acid, an important antioxidant in the protection of cells from oxidative stress; therefore dietary

intake and the recycling of ascorbate from its oxidized forms (dehydroascorbic acid and the ascorbyl free radical) are essential for maintenance of ascorbate levels. The relation between TrxR level and ascorbate cycle has been demonstrated by the observation that maintenance of rats on a Se-deficient diet results in decreased liver ascorbate, GPx and TrxR levels.⁷⁴ Interestingly, Se-containing compounds including selenite, GS-Se-SG and SeCystine are also substrates for the TrxRs, so that these Se-enzymes are themselves implicated in Se-proteins synthesis by generating selenide for assimilation.⁷⁵

Despite the wide variety of essential biological functions characterizing TrxRs, the relatively recent discovery of the isoforms TrxR2 and TrxR3 limits the knowledge of their specific role with respect to TrxR1. TrxR1 and TrxR2 are known to be essential for embryogenesis through mechanisms that appear to be non redundant.⁷⁶ The function of mitochondrial TrxR2 involves the protection from mitochondrial-mediated oxidative stress and apoptosis during embryogenesis. TrxR3 is composed of two 65 kDa subunits with an additional glutaredoxin domain. This enzyme is mainly expressed in the male germ cells and has been suggested to play a role in sperm maturation by influencing the formation of disulfide bonds in structural proteins.⁷³

4.3. Iodothyronine deiodinases

The iodothyronine deiodinases (DIOs) are a family of three integral membrane proteins with similar structure. DIO1 and DIO3 are plasma membrane proteins, whereas DIO2 is localized in the ER membrane.^{77,78} All DIOs are oxido-reductases with SeCys residue in the active site, that participate in thyroid hormone metabolism by catalyzing the activation (DIO1, DIO2) or inactivation (DIO3) of tetraiodothyroxine (T₄), triiodothyronine (T₃), and reverse-triiodothyronine (rT₃) as schematized in Fig. 6.⁷⁹ These thyroid hormones regulate various metabolic processes, such as lipid metabolism, thermogenesis, growth and hearing, that are essential for homeostasis but also for the normal development of the fetal brain.⁸⁰

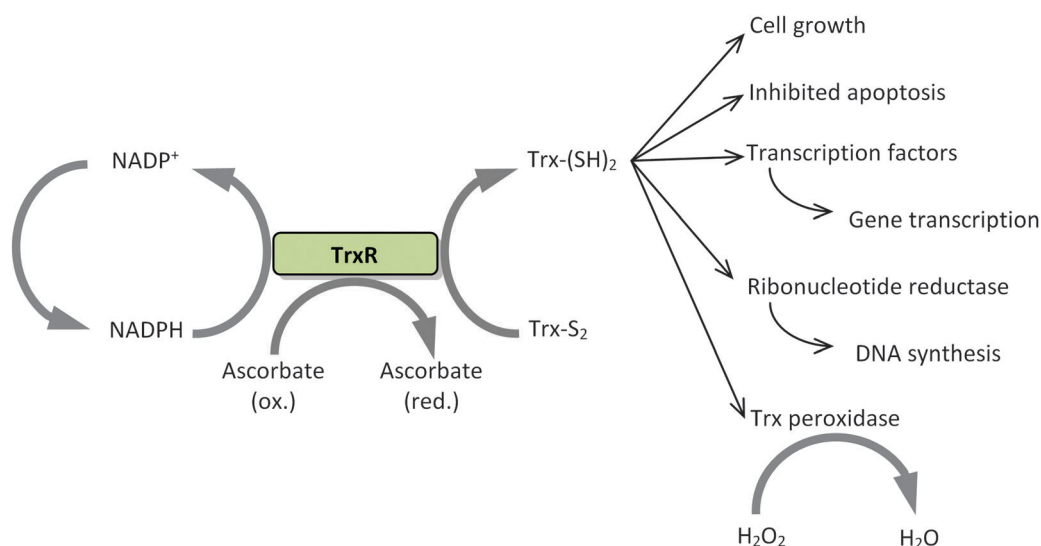


Fig. 5 Scheme of the catalytic activity and biological functions of TrxRs.

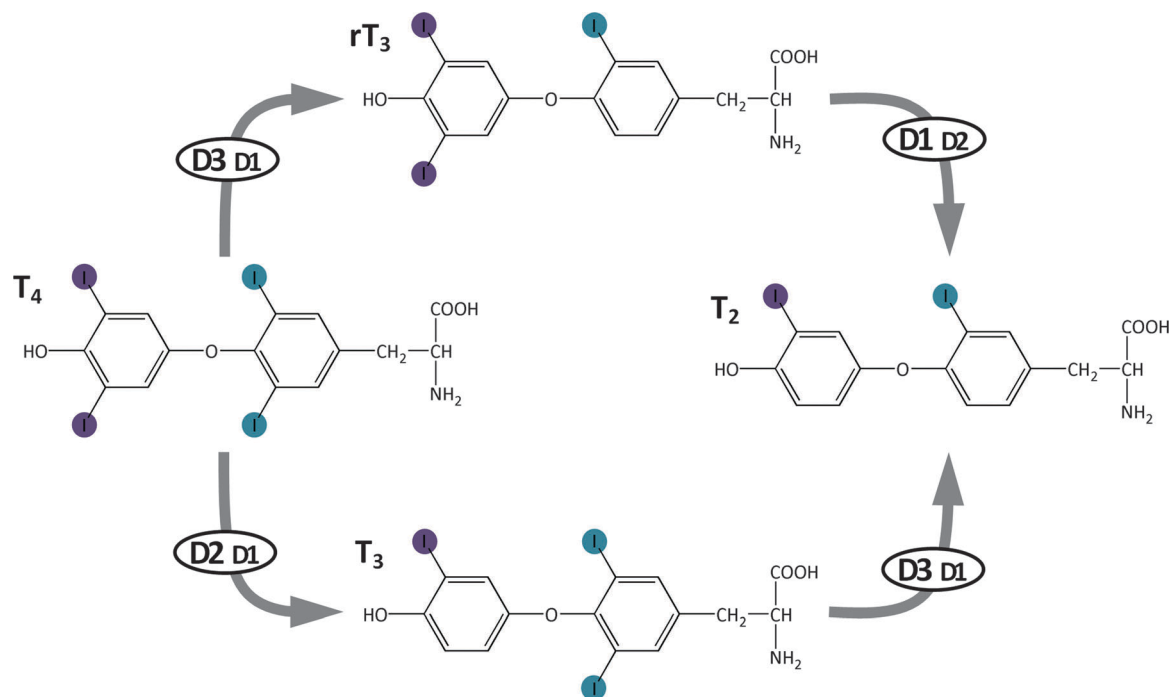


Fig. 6 Scheme of the metabolism of thyroid hormones mediated by DIOs. DIO2 catalyzes the monodeiodination of the outer ring of the iodothyronine nucleus (from T4 to T3, and from rT3 to T2), whereas DIO3 catalyzes the monodeiodination of the inner ring (from T4 to rT3, and from T3 to T2). DIO1 catalyses monodeiodinations unspecifically.

Like other Se-proteins families, the functional differences amongst individual isoforms are not yet well elucidated. The three DIOs exhibit differentiated expression patterns and tissue distribution. DIO1 is expressed mostly in the liver, kidney, thyroid, and pituitary gland; DIO2 in the thyroid, the central nervous system, the pituitary gland, and skeletal muscle.⁸¹ DIO3 presents a more specific expression pattern as it is mainly present in the embryonic and neonatal tissues. Its privation entails an abnormal developmental pattern, so that DIO3 is considered a fetal enzyme.⁸¹ It is assumed that DIO1 is responsible mainly for the control of circulating T3 levels, whereas DIO2 and DIO3 are involved in the local regulation of deiodination processes. However, their relative role in these mechanisms is still not well understood and seems to vary depending on the Se status and development stage.⁸² DIOs occupy a high rank in the hierarchy of Se-proteins for incorporation of the element under deficiency conditions, particularly concerning the accumulation and/or redistribution of DIO1 in the thyroid gland, and DIO2 and DIO3 in brain and placenta.⁸¹

4.4. Selenoproteins 15 and M

Se-proteins 15 (Sep15) and M (SelM) are thiol-disulfide oxidoreductases which constitute a distinct family of Se-proteins. In mammals, the two proteins are expressed with similar tissue distribution, Sep15 with highest levels in prostate, liver, kidney, testis, and brain, whereas SelM is mainly expressed in the brain.^{83,84} Sep15 and SelM localize in the ER; both proteins encode a N-terminal peptide which is cleaved after translocation in the ER. In addition, the native Sep15 has shown migration

properties in SDS-PAGE that are consistent with a 150–240 kDa complex, whose constituents have not yet been established.⁸⁵

Sep15 contains a Cys-rich domain in the N-terminal part of the protein, but lacks of an ER retention sequence.⁸⁶ Through its N-terminal Cys-rich domain, Sep15 has been shown to form a complex with UDP-glucose:glycoprotein glucosyltransferase (UGGT).⁸⁷ The UGGT acts as a folding sensor by initiating the association of unfolded glycoproteins with calnexin (CNX) and protein disulfide isomerase ERp57, and may also directly assist folding of a specific group of glycoproteins. The complex formed by Sep15 with UGGT is responsible for its retention by the ER and suggests a possible implication in the folding or secretion of glycoproteins.⁸⁷ Sep15 presents a thioredoxin-like domain with a surface accessible redox-active motif, Cys-X-SeCys, in which SeCys and Cys form a reversible Se–S bond.⁸⁶ In relation to its redox potential, this suggests for Sep15 a possible additional function of catalyzing the isomerization or reducing disulfide bonds.⁸⁸ Sep15 may also play a role in regulation of apoptosis, as shown in malignant mesothelioma and NIH3T3 cell lines,^{89,90} but insufficient data exists to provide the evidence.

SelM shares 31% of sequence with Sep15. Differently from the latter, SelM lacks the UGGT-binding domain and presents an ER-retention signal, whereas its redox-active motif is in the form Cys-X-X-SeCys.⁸³ The binding partners and the specific role of SelM remain to be established.

4.5. Selenoproteins S and K

The Se-proteins S (VIMP, Sels) and K (SelK) belong to the same family whose members share a short N-terminal ER luminal

sequence, an N-terminal single pass transmembrane helix, and a C-terminal active site (with SeCys in the case of Se-proteins). SelS and SelK share similar structural characteristics, binding proteins, and reaction processes involved in the regulation of ER stress, because both are transmembrane ER-resident proteins.

SelK is a ubiquitous protein, highly expressed in the spleen, immune cells, brain and heart.^{91,92} Unlike the other Se-proteins, its catalytic site is part of a motif where SeCys is not paired with a nearby Cys, Ser or Thr.⁹³ This means that a possible hydrogen bond donor for the protection of SeCys approaches the residue only as a consequence of the 3D structure of the protein, or is provided by an unknown partner. SelS presents the same motif, whose SeCys in position 188 has been recently shown to form a Se-S bond with a Cys residue in position 174.⁹⁴ The structural and redox properties of SelS and SelK suggest that they function as reductases adapted for a wide range of substrates.

SelS and SelK are involved in the ER-associated degradation (ERAD) of unfolded and misfolded proteins, a multi-step process involving many proteins whose specific roles have not been clearly elucidated.⁹⁵ Derlin-1 and -2 are strong candidates to play the role of channel proteins responsible for the retrotranslocation of unfolded proteins from the ER to the cytosol, the early stage of ERAD. Recent studies have shown that both SelS and SelK are associated with Derlins and p97 ATPase, with which may form an ER-membrane associate complex.⁹⁵ SelS may mediate the interaction of cytosolic p97 and Derlin-1,⁹⁶ but such an assumption still needs to be confirmed. Additional partners of SelS in the complex may be required to exploit its function, and SelK seems to be a potential candidate. The role of SelS and SelK in ERAD is confirmed by their upregulation under glucose deprivation,⁹⁷ and Ca²⁺ depletion,⁹⁸ both being processes inducing the aggregation of improperly folded proteins in the ER. While SelS expression is induced by ER stress, its depletion increases the release of inflammatory cytokines. SelS was also shown to interact with serum amyloid A, suggesting a potential role in type 2 diabetes which is linked to the regulation of the inflammatory response.⁹⁹

4.6. Selenoprotein W

Se-protein W (Sepw1, SelW) is a small protein with the SeCys residue as part of a Cys-X-X-SeCys redox motif localized in an exposed loop.¹⁰⁰ The analogy with the motif Cys-X-X-Cys of Trx, high affinity for GSH, and overexpression against oxidative stress in muscle tissues suggest an antioxidant function.¹⁰¹ However, the precise molecular pathways are not yet elucidated, so the specific functions remain unknown.

Recent studies have shown that SelW interacts with specific isoforms of 14-3-3 proteins.^{102,103} Such proteins participate in several cellular processes, including the regulation of the cell cycle, metabolic control, apoptosis, protein trafficking, and gene transcription. For example, SelW may play an important role in the recovery from G2 arrest, an interruption of progression into mitosis, by determining the dissociation of 14-3-3 from the phosphatase CDC25B by a redox-regulated mechanism.¹⁰²

SelW is ubiquitously expressed in tissues, with a particular preservation into the brain under Se deficiency.¹⁰⁴ In glial cells,

SelW expression is specifically induced in response to a radical generator, suggesting a potential specific function in the brain.¹⁰⁵ It is also expressed in early embryonic development, during implantation and gastrulation, and subsequently, within the nervous system, limbs, and heart.¹⁰⁶ The early developmental expression pattern of SelW in muscle progenitor cells and its high expression levels in proliferating myoblasts, suggest a specific role of SelW in muscle development and diseases.

Overexpression of SeW markedly reduces the sensitivity of Chinese hamster ovary (CHO) and lung cancer cells to H₂O₂ cytotoxicity.¹⁰¹ The SeCys residues 13 and 37 have been shown to be necessary for the antioxidant activity of SelW, which is downregulated by GSH, but seem to be not correlated with intracellular levels of reactive oxygen species (ROS). SelW was also found to be a specific molecular target of methylmercury in human neuronal cells, whereas other Se-proteins were not affected.¹⁰⁷

4.7. Selenoprotein H

Se-protein H (SelH) is a nucleolar thioredoxin-like protein with DNA-binding properties.¹⁰⁸ It is moderately expressed in various mouse tissues, whereas elevated expression levels were found in brain during early development, but also in the thyroid, lung, stomach, and liver human tumors.¹⁰⁹ These data suggest a possible role of SelH in cellular proliferation during development or cancer growth. SelH is also involved in up-regulating the levels of GSH, the activity of GPx and the total antioxidant capacity in response to the redox state, with protective effects against superoxide and cell damage induced by ultraviolet B (UVB) irradiation.^{108,110} A recent study has shown that SelH may exert its protective function through the activation of mitochondrial biogenesis signalling pathway by increasing the level of the nuclear encoded regulators PGC-1 α , NRF1 and Tfam.¹¹¹

4.8. Selenoprotein T

Se-protein T (SelT) is a member of the thioredoxin-like family that has been predicted to be a glycosylated transmembrane protein.¹¹² In mouse and rat cells it localizes in Golgi, ER and possibly in the plasma membrane.^{100,113} It is ubiquitously distributed, with high expression in the testes.⁵⁸ The expression of SelT is regulated by the trophic neuropeptide pituitary adenylate cyclase-activating polypeptide (PACAP).^{113,114} Elevated expression was found in embryonic tissues, followed by a decrease in most adult tissues, excluding the pituitary gland, thyroid and testis.¹¹⁴ SelT was found to be highly expressed also in the brain of hypoxia-induced mice¹¹⁵ and in regenerating liver cells after partial hepatectomy.¹¹⁴ Altogether these observations suggest an important role for SelT in ontogenesis, tissue maturation/regeneration, and cellular metabolism of nervous and endocrine tissue, with a possible redox action in Ca²⁺ homeostasis.¹¹³ Structural analogies characterize SelT and SelW, indicating a potential functional relation, supported by the observation that knockdown of SelT in mouse fibroblasts may be compensated by increased expression of SelW.¹¹⁶

4.9. Selenoprotein V

Selenoprotein V (SelV) is a member of thioredoxin-like family exclusively expressed in spermatocytes.¹ It has a proline-rich N-terminal domain and SeCys located in a hydrophobic domain. Possible partners for SelV have been recently proposed, including the proteins *O*-acetylglucosamine transferase (OGT), Asb-9 and Asb-17; the Asb-family proteins present the SOCS domain, a suppressor of cytokine signalling.^{117,118}

4.10. Selenoprotein P

Se-protein P (SEPP1, SelP) is the only Se-protein containing ten SeCys residues in rats, mice, and humans.² It is a glycosylated protein since it presents 3 occupied N-glycosylation sites and 1 occupied O-glycosylation site.¹¹⁹ Several disulfide and selenenyl-sulfide bonds have also been identified in purified rat SelP. These bonds might have structural functions and might serve in the protection of the reactivity of selenolic groups.¹¹⁹ SelP purified from rat plasma is present as 4 isoforms containing a lower number of 6 SeCys residues.¹²⁰ A recent study reported the separation and characterization of three distinct SelP isoforms also in human plasma, with Mw of 45, 49 and 57 kDa, the first being a truncated isoform lacking in Se.^{121,122} SelP is mainly produced in the liver and then secreted into the plasma, where it incorporates the major part of Se, but it is expressed and probably secreted also by other tissues including the brain and the heart. As mentioned in Section 3.3, evidence supports functions of SelP in Se transport and homeostasis throughout the whole body.¹⁸ SelP knockout mice present very low Se concentrations in brain, testis and foetus, with severe pathophysiological consequences in each tissue.¹²³ In addition, these mice excrete moderate amounts of Se in the urine. On the other hand, dietary Se deficiency causes a profound decrease in liver Se concentration, presumably because the liver exports a fraction of its metabolically available Se as SelP, even when the element supply to the organ is drastically decreased. Under dietary Se deficiency, SelP appears to be responsible for maintaining preferentially Se in the brain and testis by a mechanism that is distinct from its effect on the other organs.^{124,125} In the kidney and in the muscle, Se concentration falls approximately to the same extent as does whole-body Se with the deletion of SelP and with dietary deficiency. Since Se is covalently bound, its release requires disruption of SelP to exploit its transport function.¹²⁶

The specific biochemical activity of SelP remains still unclear. Indications exist about the possible role of the protein in antioxidant defence. The SelP plasma level correlates with prevention of lipid and low density lipoproteins peroxidation and hepatic endothelial cell injury, and an association has also been reported between SelP and protection against oxidant injury from GSH depletion in Se-deficient rats.^{18,127} In addition, SelP binds to endothelial cells in the rat, probably through its heparine-binding site.^{18,128} Endothelial cells release primary free radicals NO[•] and O₂[•] from which peroxynitrite (ONOO[−]) and H₂O₂ secondary products are formed. Thus, endothelial cells and their environment have been hypothesized to be sites

of oxidative stress. Localization of SelP in proximity of endothelial cells is consistent with its having an antioxidant defence function related to the protection of membranes.

4.11. Selenophosphate synthetase

As mentioned earlier, selenophosphate synthetase 2 (SPS2) catalyzes the synthesis of SePhp, the key Se-donor for Se-proteins biosynthesis, by transferring the γ -phosphoryl group of ATP to selenide.⁴⁷ Two isoforms of SPS (1 and 2) are encoded in higher eukaryotes, but SPS1 is not a Se-protein since the SeCys residue is replaced by Arg. Despite a study has shown that SPS1 catalyzes SePhp biosynthesis very weakly and using only SeCys as substrate, suggesting a role in recycling process of SeCys,¹²⁹ a further work did not confirm this hypothesis.¹³⁰ SPS1 seems to be unnecessary for the assimilation of Se in Se-proteins, but may play another role in Se metabolism as shown by its potential to complex with a number of proteins involved in SeCys biosynthesis.¹³¹ The Se-protein SPS2 remains the only responsible for the generation of SePhp, but the exact mechanism driving this reaction has yet to be determined.

4.12. Selenoprotein R

Se-protein R (MsrB1, SelX, SelR) belongs to the methionine-sulfoxide reductases family. These proteins are responsible for the reconversion of Met residues from their oxidized form methionine sulfoxide (MetSO), which can negatively affect a number of biological functions.¹³² Methionine sulfoxide forms by the action of ROS as a mixture of two diastereomeric forms (Met-S-SO and Met-R-SO), in which reduction is specifically mediated by distinct enzyme families, respectively named MsrA and MsrB. At least four different MsrB products, encoded by specific genes, have been identified in humans, each with a proper subcellular location: MsrB1 in the cytoplasm and nucleus, MsrB2 and MsrB3B in mitochondria, MsrB3A in the ER.¹³³ However, only MsrB1 is a Se-protein, whereas a Cys residue substitutes SeCys in the active site of the other three products. The catalytic activity of MsrB1 proceeds through the steps which are schematized in Fig. 7.¹³⁴ Firstly, the SeCys attacks the substrate to form methionine and is converted into a selenenic acid intermediate. In the following step the recycling Cys attacks the selenenic acid to form a Se-S bond, which is further reduced by Trx. Interestingly, SelR is also a zinc-containing enzyme.¹³⁵ The metal is bound through four Cys residues, and has been suggested to play a structural function.¹³⁶

4.13. Selenoprotein N

Se-protein N (SelN) is a ubiquitous glycoprotein highly expressed in fetal tissues, muscle, brain and lung.¹³⁷ The catalytic site consists of the motif Ser-Cys-SeCys-Gly, similar to that of TrxR (Gly-Cys-SeCys-Gly), so that a reductase-function may be hypothesized. However, limited access to the site, located in the centre of the protein, and the absence of typical FAD- and NADPH-binding domains, may reflect the higher specificity of SelN for different substrates, that are not yet identified.¹³⁸ SelN localizes in the membrane of the ER, with the N-terminal region facing the cytoplasm, while the bulk of the protein,

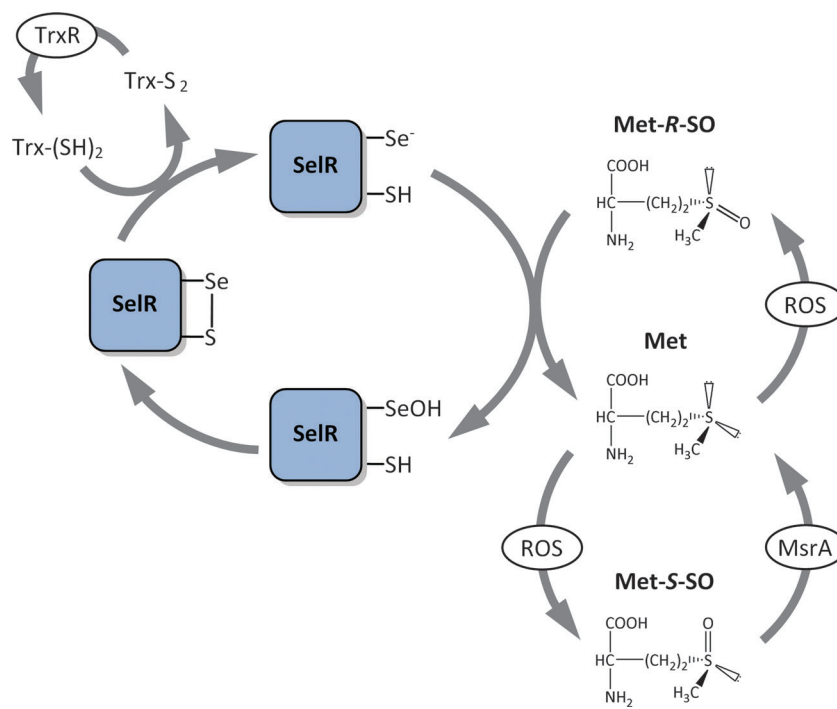


Fig. 7 Scheme of the reduction of methionine-sulfoxide catalysed by SelR.

including the potential active site, resides within the lumen of the ER.^{137,138} A recent study has shown that SelN co-localizes and co-immunoprecipitates with the ryanodine receptor (RyR), a component of the intracellular Ca^{2+} release channel.¹³⁹ Also, SelN modulates the activity of RyR and protects it against oxidative stress. The association with Ca^{2+} release links SelN to a potential function in the development of slow muscle fibers in embryos.¹⁴⁰

Despite its confirmed key role in muscle tissue, the specific biological function of SelN remains unknown. Paradoxically, it is the only Se-protein whose connection to a disease, SEPNI-related myopathy, has been directly established as a consequence of mutations in the SelN gene. This connection is discussed in Section 5.2.

4.14. Selenoprotein I

The Se-protein I (SelI), also named ethanolaminephosphotransferase 1 (hEPT1), is a recently discovered protein which participates to the biosynthesis of phosphatidylethanolamine (PE).¹⁴¹ PE resides in the inner leaflet of plasma membrane, where it constitutes ~25% of the whole pool of cellular phospholipids in mammals. This phospholipid is an important precursor of the glycosylphosphatidylinositol anchors and of *N*-acylethanolamine, a neurotransmitter in the brain, and is involved in membrane fusion events and proteins folding.¹⁴² One of the two possible routes for the biosynthesis of PE is the CDP-ethanolamine (Kennedy) pathway (the other route is mitochondrial decarboxylation of phosphatidylserine, PS). The final step of the pathway, the transferring of phosphoethanolamine from CDP-ethanolamine to diacylglycerol, is catalyzed by choline/ethanolaminephosphotransferase 1 (CEPT1), an enzyme that

can also use CDP-choline as a substrate for the synthesis of phosphatidylcholine (PC). It was originally thought that CEPT1 is only responsible for the biosynthesis of PE *via* the Kennedy pathway. However, it has been shown that SelI may participate in the process, exhibiting a specific affinity for CDP-ethanolamine. SelI is ubiquitously expressed, with particularly high abundance in the cerebellum.¹⁴¹

4.15. Selenoprotein O

Selenoprotein O (SelO) has been identified as the largest mammalian Se-protein, and is one of the most obscure human proteins.¹ A double function of kinase signalling and redox detection/signalling has been recently predicted for the generic family of SelO-like proteins,¹⁴³ but no more specific structural and functional characterizations are available.

5. Selenium and human diseases

It has been proven in the last two decades that Se may be directly or indirectly linked to a large variety of human health disorders. Most of these associations are due to the role of GPxs and TrxRs enzymes in the reduction of oxidative stress, which has been identified as a main cause in the development and progression of several pathologies. Some other Se-proteins are involved in specific processes such as Ca^{2+} signalling, brain function and spermatogenesis. Alterations in their genes or underexpression related to Se deficiency have been identified as possible causes of the corresponding pathology. However, definitive knowledge concerning the mechanisms underlying the action of Se-proteins related to human diseases is still far from being reached. Apparently conflicting data arise from the large

Table 2 Hypothetical role of normally active Se-proteins with respect to human diseases. Evaluation *a priori* must always consider Se-proteins as concurrent factors. An inhibitory effect implies a direct action within the disease generating processes, whereas mitigation refers to posterior reduction of damage. Given each role under normal activity, the contrary effect arises when the activity of the protein is sub/supra-normal

Disease	Se-protein	Role	Mechanism
Muscle disorders	SelN, SelW	Prevention	Homeostasis of Ca ²⁺ signalling
Cardiovascular diseases	GPxs, TrxRx, SelR	Prevention/mitigation	Antioxidant defence
	DIO1	Prevention/mitigation	T3 hormone supply for lipid metabolism
	Sels	Prevention	Unknown
Hepatopathies	GPxs	Mitigation	Antioxidant defence
Renal failure	GPxs	Prevention/mitigation	Antioxidant defence
Epilepsy, mood disorders	GPxs	Prevention	Antioxidant defence
Neurological disorders (other)	SelP, GPxs, TrxRs, SelW, SelH, SelM	Mitigation	Antioxidant defence
Inflammatory response	TrxRs	Promotion	Early regulation of immune cell signalling
	GPxs	Inhibition	Advanced regulation of immune cell signalling
	Sels	Inhibition	Antioxidant defence, cytokine regulation
HIV	GPxs, others	Mitigation	Antioxidant defence
Type 2 diabetes	GPxs	Promotion/mitigation	Inhibition of the insulin signalling/antioxidant defence
	SelP	Promotion	Inhibition of insulin synthesis
	TrxRs	Prevention/mitigation	Stimulation of the insulin signalling/antioxidant defence
Endocrine disorders	DIOs	Prevention	Regulation of thyroid hormones metabolism
Male infertility	GPx4	Prevention	Antioxidant defence, structural support
Cancer	GPxs, SelP, TrxRs	Prevention	Antioxidant defence

number of epidemiological investigations where the total Se concentration in food/supplements, blood fractions and toenails was assessed in relation to the onset or progression of pathological status. Conversely, a wide range of biochemical information has been collected on specific cellular processes involving Se and Se-proteins. Finding causal connections between the cell and population levels, passing through the individual is a major challenge. Table 2 summarizes up to date information on the role of Se-proteins in human health and the correlation of their alteration with several human diseases. Each action refers to the protein when present in normal activity, in such a way that the opposite role can be inferred when the activity of the protein is sub/supra-normal. Most of the Se-proteins exhibit a beneficial action with respect to human diseases, meaning that a deficient activity may be associated with the occurrence or progression of the pathological state. However, it is important to emphasize that the role of individual proteins has to be contextualised within a complex biochemical environment, where antagonistic, additive and synergistic effects take place. An example is given by the balancing action of TrxRs and GPxs for the modulation of immune response and glucose cellular uptake.

5.1. Deficiency and toxicity

Severe Se deficiency is directly associated with two endemic diseases diffused in soil Se-poor regions of China and Russia: Kashin-Beck and Keshan diseases. Kashin-Beck disease is an osteoarthritis characterized by atrophy, degeneration, and necrosis of cartilage tissue, which occurs primarily in children between the ages of 5 and 13 years. The pathology results in enlarged joints, shortened fingers and toes, and dwarfism in extreme cases.^{28,144} Keshan disease is a muscular disorder and is discussed in Section 5.2.

Acute Se toxicity by inhalation exposure causes stomach pain and headaches, and a number of respiratory symptoms

such as pulmonary edema, bronchial spasms, symptoms of asphyxiation and persistent bronchitis, elevated pulse rates, lowered blood pressure, vomiting, nausea, and irritability.¹⁴⁵ Acute oral exposure to extremely high levels of Se provokes nausea, vomiting, diarrhoea, and occasionally tachycardia. Regarding chronic inhalation exposure, a number of occupational studies revealed respiratory effects such as irritation of the nose, respiratory tract, and lungs, bronchial spasms, and coughing.¹⁴⁵ Chronic oral intake of very high levels of Se results in selenosis, a specific pathology characterized by hair loss, deformation and loss of nails, discoloration and excessive decay of teeth, garlic breath, gastrointestinal disturbances, skin rash, and abnormal functioning of the nervous system (numbness, paralysis and occasional hemiplegia).^{146,147} Related toxic effects are the disruption of the endocrine function, synthesis of thyroid hormones and growth hormones, and insulin-like growth factor metabolism. Particularly high levels of dietary Se are also significantly associated with impairment of natural killer cells and hepatotoxicity.¹⁴⁶

5.2. Muscle disorders

Keshan disease is an endemic juvenile cardiomyopathy with myocardial insufficiency, that primarily affects children between 2 and 10 years old.²⁸ This pathology is characterized by cardiac enlargement, abnormal electrocardiogram (ECG) patterns, cardiogenic shock, and congestive heart failure, with multifocal necrosis of the myocardium.¹⁴⁵ Selenium deficiency was identified only in the 1970s as being the major cause of Keshan Disease. Evidence was firstly based on extensive observational epidemiological studies carried out in northeast and southwest areas of China, where the disease was endemic.¹⁴⁸ A strong association was revealed between the occurrence and geographic distribution of the disease with low Se intake, and blood Se status and GPxs activity in patients. Intervention trials were conducted by administering sodium selenite to large

population samples, resulting in a significantly reduced incidence of this disease.

The mechanism linking Se deficiency and Keshan disease is not completely understood. Biochemical and clinical studies suggest that a decreased activity of GPxs (particularly GPx1) related to Se deficiency may impair the protection of mitochondria against membrane peroxides-induced damage.¹⁴⁹ Further studies have shown that Se deficiency is not sufficient to fully explain the incidence of Keshan disease.¹⁵⁰ Rather, its etiology is triggered by a combination of Se deficiency and infection by the enterovirus *Coxsackie*: insufficient Se intake impairs the antioxidant action of Se-proteins, so viral DNA is exposed to oxidative damage, which increases its virulence.

Muscular dystrophy is another group of pathologies involving the slow degeneration of muscle tissue.¹⁵¹ Some forms of congenital muscular dystrophy, including multiminicore myopathy, rigid spine muscular dystrophy and desmin-related myopathy with Mallory bodies, have been linked to mutations of the SelN gene (*SEPN1*). All these pathologies share clinical features and are referred to as *SEPN1*-related myopathies. However, the role of SelN in muscular dystrophy has been elusive because its biological function is still largely unknown. The observed association of SelN with ryanodine receptors, that are responsible for Ca²⁺ signalling, may provide an explanation.¹³⁹ Mutations in *SEPN1* prevent this association, and thus may be responsible for multiminicore disease by the inhibition of Ca-stimulated release of Ca²⁺ from intracellular stores.¹⁵² However, as long as the biological function of SelN remains obscure, no more conjecture can be proposed.

SelW has also been linked to muscular functions since a lower concentration of this protein has been observed in animals affected by white muscle disease (WDM).¹⁵³ As for SelN, such an association may be due to a role of the Se-proteins in the regulation of Ca²⁺ homeostasis, because the sarcoplasmic reticulum of WDM animals exhibits a defective Ca²⁺ sequestration, resulting in the calcification of skeletal and cardiac muscles.¹⁵³ A possible action of SelW in the regulation of Ca²⁺ metabolism was originally proposed, but the hypothesis has not been demonstrated at a later stage, and no link to humans has been documented.

5.3. Cardiovascular diseases

Oxidative stress damages the vascular endothelial cells and exacerbates cardiovascular diseases (CVD) such as atherosclerosis, hypertension, and congestive heart failure.¹⁵⁴ Since most Se-proteins are involved in the cellular antioxidant defence system, a potential prevention effect of adequate Se intake has been hypothesized for non-infectious CVD. This topic has been investigated by a large number of epidemiological studies based on both observational data and clinical trials, without reaching a conclusive response. The general CVD incidence in supplementation trials has been recently reviewed considering twelve research publications, revealing no significant association with Se intake.¹⁵⁵ Two meta-analyses observational studies were also conducted on blood, serum/plasma or toenail Se levels and compared with the incidence of coronary heart

disease (CHD); a moderate inverse correlation between total Se concentration and CHD risk was found.^{156,157} However, a clear causal connection cannot be inferred under the observed differences, mainly because of the potential confounding effect of the other co-supplemented antioxidants. Similar shortcomings also affect randomized trials carried out to assess the effects of Se supplementation on CHD,¹⁵⁶ as well as most of the studies in the literature.¹⁵⁸

Apart from possible preventive actions, Se-proteins may play a more evident role in cellular defence against ROS production during or after the development of CVD. A positive correlation was observed between GPx3, TrxR1 and SelR, with ROS production in heart hypertrophy.¹⁵⁹ The function of TrxRs in CVD may pass through the modulation of Trxs, that in turn regulates the response to ventricular remodelling after myocardial infarction.¹⁶⁰ Conversely, Se supplementation, resulting in higher GPxs and TrxRs activity, has been shown to reduce the oxidative damage after cardiac ischemia-reperfusion.¹⁶¹

Another Se-protein associated to CVD through a more specific action is DIO1.¹⁶² This protein controls the conversion of T4 into T3, which is the active form of thyroid hormone and plays a key role in normal lipid metabolism. Hypothyroidism causes qualitative changes in circulating lipoproteins, increasing their atherogenicity. Thus, an adequate activity of DIO1 during hypercholesterolemia is particularly important to preserve the homeostasis of lipid metabolism through the efficient supply of T3.

SelS was found to be associated with CVD, carrying out a protective effect on astrocytes during ischemia, but its mechanism of action is unknown.¹⁶³ Finally, variations in the *SEPS1* locus are associated with CHD risk in females.¹⁶⁴

5.4. Hepatopathies

Hepatopathies are another family of disorders that have been linked to high levels of oxidative stress, in which antioxidant enzymes may play beneficial actions.¹⁶⁵ The liver pathology provoked by alcoholism is characterized by an infiltration of leukocytes and formation of collagen in hepatocytes. This process is driven by increased production of free radicals, resulting in lipidic peroxidation of the cell membranes. The actions of ethanol-cytochrome P450 3E1 and aldehyde oxidase on ethanol and acetaldehyde, respectively, generate superoxides. These ROS produce hydroxyl radicals which can directly react with ethanol, generating 1-hydroxyl-ethyl radicals. In this context, it has been proposed that antioxidant enzymes such as GPxs could play an important role to oppose the augmented ROS entailing high alcohol intake.¹⁶⁵ Several studies investigated the total Se level in the liver, whole blood, erythrocytes, plasma or serum of patients with hepatopathies, most of them finding lower values with respect to control subjects.⁹ Recent works have confirmed this observation for cirrhotic patients,^{166,167} and have also found an association between depleted Se status and increased mortality.¹⁶⁸ No additional correlation is documented between serum Se and severity of the disease.¹⁶⁷ Considering these data, many authors suggested that Se supplementation may improve the hepatic functionality

in the treatment of cirrhosis, an effect confirmed by recent randomized trials.^{166,169}

Despite the fact that serum Se may be partially related to structural liver dysfunction, evidence supports that low Se in alcoholic individuals is mainly due to a low nutritional supply of the element because the excessive intake of alcohol is accompanied by a decrease in the consumption of other food.^{168,170} This provides additional support to the potential benefit of Se supplementation and general antioxidant therapy in the care of hepatic diseases caused by alcoholism.

5.5. Renal failure

Plasma GPx3 activity and Se level in RBCs, whole blood or plasma were found to be significantly lower in patients with chronic renal failure (CRF) and in hemodialyzed (HD) uremic patients compared to healthy controls, in some cases it has also been associated with progression of the disease.^{171–173} Decreased dietary intake of Se, increased urinary (or dialytic) loss, impaired intestinal absorption, abnormal binding to Se transport proteins and other mechanisms have been proposed to explain this association, but conclusive results are still missing. Since the circulating levels of Se are low in CRF and HD patients, Se supplementation could lead to positive effects, as recently demonstrated for immune function improvement and oxidative stress reduction,¹⁷⁴ at least in some cases, but may be insufficient in others. For example, Se supplementation was found to not increase the activity of GPx3 in CRF patients, showing that a basic impairment exists in the ability of the kidney to synthesize this Se-protein.¹⁷⁵ Other factors such as dialysis and treatment procedures for renal failure have a controversial relationship to Se levels. Starting from low Se status in all cases, some authors have found that dialysis contributes to a drop of blood Se at a grade which depends on the type of membrane used,¹⁷⁶ whereas other authors have observed an increase of plasma Se level and GPxs activity after dialysis.¹⁷⁷ Collateral treatments are also relevant, *i.e.* the low Se level in blood could be reversed by treating HD patients with statins (that have anti-inflammatory and antioxidant properties).¹⁷³ More studies are needed to elucidate the role of specific factors in interaction with the altered Se metabolism that characterizes uremic patients. Similarly to other diseases, a decreased Se level may be a direct or indirect consequence of renal failure, but could also exacerbate the oxidative damage, enhancing the susceptibility to complications occurring in CVD.¹⁷⁸

5.6. Neurological disorders

Selenium distribution within the different regions of the brain appears to follow a specific scheme, characterized by higher levels in the regions with abundant grey matter and in the glandular parts.¹⁷⁹ Brain Se showed an exceptional tendency to be preserved into the organ under conditions of dietary deficiency,¹⁸⁰ whereas knockout of the supply routes is accompanied by the onset of severe neurological dysfunction.¹⁷⁹ These observations suggest important roles for Se and Se-proteins in the brain, which is also highly exposed to

oxidative stress due to elevated oxygen consumption. Damage from ROS takes place in several neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, ischaemic damage, exposure to environmental toxins, abuse of drugs, brain tumors, multiple sclerosis, Batten's disease and epilepsy.^{181,182} Considering the antioxidant action carried out by many Se-proteins, these species are of potential interest as disease biomarkers in neurological disorders.

Oxidative damage to macromolecules is an early indication of Alzheimer's disease (AD) that can appear before clinical symptoms.¹⁸³ The brain of AD patients is characterized by intracellular neurofibrillary tangles and extracellular plaques consisting of the protein amyloid β . Both features have been observed in mice with genetic deletion of SelP, together with impairment of synaptic function in the hippocampus, a region involved in memory, and the reduction of spatial learning and long-term potentiation, a cellular model for learning and memory.¹⁸⁴ SelP presents a characteristic expression pattern within the centre of neuritic (dense-core) plaques.¹⁸⁵ Although a specific action of SelP in AD is still uncertain, its distribution in the brain suggests a role in mitigating the oxidation accompanying plaques. The age-related alteration of other Se-proteins activity in brain of AD patients results in increased oxidative stress and reduced protection against neurodegeneration through redox regulation. Other Se-proteins including GPx1, GPx4, TrxR1, SelW, SelH and SelM may be involved in these functions.¹⁸² Most of them are up-regulated in response to brain injury and ROS exposure and can be considered necessary for the maintenance of redox homeostasis in the brain, but their specific mechanism of action is unknown.

Parkinson's disease (PD) is another well studied neurodegenerative disorder. It is characterized by severe loss of dopamine-releasing neurons in the *substantia nigra*, where particularly high levels of Se were observed under normal conditions. Se deficiency was proven to exacerbate the chemical lesions of dopaminergic terminals and neurons in PD mouse models,¹⁸⁶ whereas Se supplementation and over-expression of GPx1 have a protective action.¹⁸⁷ However, the role of GPxs and other Se-proteins in protecting dopaminergic transmission and preventing PD is still unsupported by the evidence of a direct correlation between proteins expression or function and PD.¹⁸⁸

Epilepsy, ischaemia and brain trauma cause a signal cascade of free radicals and activation of pro-apoptotic transcription factors, resulting in neuronal loss.¹⁸⁹ So, these pathologies could also be associated with altered Se-proteins activity in ROS reduction. As a support, GPx1 activity appears to be correlated with induced seizures in mice.^{190,191} SelP-knockout mice develop neurological seizures and movement disorders on a Se-deficient diet, providing further evidence for the possible role of Se-proteins in the prevention of epilepsy.¹²⁵

Potential alterations in Se status and Se-proteins activity are expected to be investigated in the near future in relation to many other neuropsychiatric disorders. For example, lower levels of total Se and GPx activity were found in obsessive-compulsive disorder,¹⁹² while a reduced Se intake was identified as risk factor for the development of major depressive

disorder (MDD).¹⁹³ Mood was also shown to be potentially conditioned by Se intake, so the influence of Se on brain functions may take place at a very essential level.¹⁹⁴

5.7. Immune defence and inflammatory disorders

The immune system relies on many processes including the generation of ROS as a defence against microbial pathogens, coordinated regulation of adhesion molecules and the expression of soluble mediators such as eicosanoids and cytokines and their receptors. Se influences immunity through many mechanisms, which have recently been reviewed in detail.¹⁹⁵ As a part of the antioxidant system, GPxs and TrxRs contribute to control ROS when they are produced in excessive concentrations during an immune reaction. The same Se-proteins also coparticipate to a complex redox equilibrium that modulates immune cell signalling.

GPxs may function as a secondary messenger in leukocyte activation by mediating the action of H₂O₂.¹⁹⁶ According to conventional theory, H₂O₂ acts directly as signalling molecule for the oxidation of adjacent Cys residues and formation of disulphide bonds in proteins with redox-regulated Cys residues, resulting in a change of their activation state. In this context, the depletion of H₂O₂ by GPxs may interrupt the signalling process. Conversely, a recent model proposed that under specific conditions the oxidized GPxs may promote the formation of disulfide bonds in regulated proteins.¹⁹⁶ As a whole, this complex mechanism modulates the activation/deactivation of important signalling proteins involved in the immune response, for example protein tyrosine phosphatases (PTPs).

In addition, in T cells TrxRs mediate the reduction of disulphide bonds through Trx.¹⁹⁷ Free thiols stimulate the efficacy of T cell receptor (TCR) -induced signals including Ca²⁺ flux and nuclear factors of activated T cells (NFAT), which are the processes involved in the generation of oxidative burst and in the regulation of cytokines.

Thus, GPxs and TrxRs play complementary roles where equilibrium is a key factor in the modulation of immune response. Studies carried out on Se or GPx1-deficient T cells marked this equilibrium: compared to normal T cells, the lack of GPx1 resulted in increased interleukin-2 receptor (IL-2) expression and interferon- γ (IFN- γ) production (this enhances the oxidative burst), according to the action of TrxR1, whereas Se deficiency resulted in the opposite effect, according to globally reduced Se-proteins.^{197,198} This picture of the redox regulation suggests that in T cells TrxRs stimulate the early TCR signalling events, whereas GPxs are devoted to limiting the extension of the inflammatory response after TCR signalling.

Another specific Se-protein involved in the immune response is SelS. Its expression in liver cells is regulated by inflammatory cytokines and extracellular glucose concentration.¹⁹⁹ SelS has an antiapoptotic role, and reduces ER stress in peripheral macrophages.²⁰⁰ A particular polymorphism of SelS was proven to be responsible for increased plasma level of the inflammatory cytokines.²⁰¹ A possible increased risk of several inflammatory diseases could be the consequence, but a direct correlation with stroke, autoimmune disorders or inflammatory bowel disease is still not proven.¹⁸⁸

As a whole, Se participates in the immune response through several actions: it regulates the balance of activity in the eicosanoid synthesis pathways, leading to preferential synthesis of leukotrienes and prostacyclins over thromboxanes and prostaglandins, and down-regulates cytokine and adhesion molecule expression.²⁰² By up-regulation of the interleukin-2 receptor expression, it leads to enhanced activity of both T and B lymphocytes, natural killer and lymphokine activated killer cells. Mice with a T cell-specific deletion in tRNA^{[Ser]Sec} resulted in knockout of all Se-proteins in the T cell.²⁰³ This produced many effects, including decrease in their functionality, reduced antigen-specific production of immunoglobulins *in vivo*, moderate to severe atrophy of the thymus, spleen and lymph nodes. Se-deficient mice exhibit increased pathology from viral infection, owing to an exaggerated pro-inflammatory immune response.^{204,205} Se deficiency or deletion of GPx1 in mice also increases viral mutations and virulence.²⁰⁶

Accordingly from these functions, it is expected that Se carries out beneficial effects on inflammatory conditions. Negative correlations were observed between serum Se level and rheumatoid arthritis, asthma, and immune activation (through soluble interleukin-2 receptor and erythrocyte sedimentation rate) in Crohn's disease.^{207–209} Plasma Se and SelP concentration is lower in patients with sepsis at different levels of seriousness or sepsis-like illness.²¹⁰ Blood, plasma or erythrocytes Se level is generally lower in patients with psoriasis, a chronic immune-mediated skin lesion.²¹¹ However, neither the reason for such a decrease and its mechanisms are known with certainty. Intervention studies have been carried out in patients with severe sepsis, suggesting potential benefits for the clinical outcome, but confirmatory data based on large populations are needed.¹⁹⁵

5.8. HIV

The implications of Se for the immune system have stimulated the investigation of its role in HIV contrasting. Chronic oxidative stress has been reported during the early and advanced stages of HIV-1 infection, and has been linked to HIV-induced apoptosis of T cells, development and progression of AIDS, Kaposi sarcoma, and related neural damage.^{212,213} Several studies on Se status and HIV-1 progression observed a direct association between low plasma/serum Se concentration or erythrocytes GPx1 activity, and reduced CD4+ counts, progression from AIDS to HIV and mortality.²¹⁴ Nevertheless, other studies have not found relatively low serum Se in HIV-1-infected subjects, suggesting that its deficiency in HIV-1 infection may be most likely to occur in subjects with poor diets, such as intravenous drug abusers and those living in poverty.^{215,216} Thus, maintaining an optimal Se status in HIV-1 patients may help to increase the enzymatic defence, improve general health and reduce their risk of hospitalization for opportunistic infections and complications.²¹⁷

5.9. Diabetes

The association between Se and type 2 diabetes involves several mechanisms, schematized in Fig. 8. Type 2 diabetes is characterized by defects in insulin secretion and action caused by

inability of the body cells to respond to the presence of insulin (insulin resistance). Binding of insulin to its receptor initiates a signalling cascade which induces a mild oxidative burst where H_2O_2 acts as a secondary messenger.⁷ Hydrogen peroxide oxidizes redox regulated Cys residues, leading to the deactivation of tyrosine phosphatase 1B (PTP-1B) and phosphatase and tensin homolog protein (PTEN). PTP-1B deactivates the insulin receptor substrate (IRS), whereas PTEN inhibits the phosphatidylinositol 3-kinase (PI3K), resulting in the overall stimulation of signalling pathway for glucose uptake. GPx1 and GPxs reduce H_2O_2 , so they are supposed to carry out an inhibitory action on the signalling cascade. Experimental evidence supports this hypothesis because transgenic mice over-expressing GPx1 exhibit insulin resistance,²¹⁸ whereas knockout of GPx1 improves insulin sensitivity.²¹⁹ Confirmation in humans raised from the observation that increased erythrocyte GPx1 activity associates with mild insulin resistance in pregnant women,²²⁰ and systemic Se-proteins deficiency (by mutations into the SBP2 gene) enhances insulin sensitivity.²²¹

However, other Se-proteins participate in glucose metabolism, making the global effects of Se more complex. SelP is supposed to inhibit the insulin signalling pathway by inactivating the adenosine monophosphate-activated protein kinase (AMPK), a positive regulator of insulin synthesis in pancreatic insulin-producing β cells.²²² *In vitro* studies demonstrated also that SelP expression in human subcutaneous adipocytes is up-regulated by insulin.²²³

TrxRs may positively influence insulin signalling by providing reducing equivalents in the form of Trx. In skeletal muscle, proteins S-nitrosylation operated by NO has been proposed to contribute to the induction of muscle insulin resistance.²²⁴ S-Nitrosylation of the subunit β of IR and Akt attenuates their kinase activities, and S-nitrosylation of IRS-1 reduces its expression, resulting in the inhibition of glucose uptake.²²⁵ Trx and its recycling, mediated by TrxR1, play an important role in the regulation of this cellular process by reducing NO.²²⁶ Additionally, both Trx1 and GSH (that is regulated by GPx) in the disulfide form can be nitrosylated and subsequently *trans*-nitrosylate proteins, thus functioning either to denitrosylate

or nitrosylate proteins depending on their redox state.²²⁷ So, the overall effect of Se level in the context of insulin signalling under normal conditions is arduous to extricate.

In addition, hyperglycaemia induces oxidative stress through activation of the polyols pathway, which increases the utilization of NADPH and the production of superoxide anions. The toxicity of high glucose levels is also related to free radicals generated by auto-oxidation of sugars, prostanoids metabolism, and proteins glycation. A consistently high oxidative stress level or low antioxidant defence were revealed in patients with diabetes, which are responsible for many pathogenic processes of diabetic complications.²²⁸ As for some other pathologies, a general protective function of Se may rely on the action of Se-proteins for ROS reduction. Several studies demonstrated that Se prevents or alleviates the adverse effects that diabetes has on cardiac and renal functions, vascular complications, and atherosclerosis progression.^{229,230}

Observational studies on Se supplementation have shown that the element can have insulin-mimetic properties, being effective in the stimulation of glucose uptake both *in vitro* and *in vivo*, the regulation of glycolysis, gluconeogenesis, fatty acid synthesis and the pentose phosphate pathway.²³¹ Selenate in particular has been proposed to influence two important mechanisms involved in insulin resistance: firstly it reduces the activity of liver cytosolic protein tyrosine phosphatases (PTPs) as negative regulators of insulin signalling; and secondly it increases the expression of the peroxisome proliferator-activated receptor gamma (PPAR γ).²³² These two mechanisms are responsible for the changes in the intermediary metabolism, in particular gluconeogenesis and lipid metabolism.

Several case-control and randomized trials have been conducted to investigate the possible direct association between the Se status and the incidence of type 2 diabetes, resulting in apparently contradictory results. Some studies have shown that mean plasma, serum or blood total Se concentrations, GPx3 and SeAlb level, or GPx3 activity are lower in patients with diabetes than in controls.^{233–236} Lower plasma/serum Se levels have also been found in gestational diabetic pregnancies with respect to normal pregnancies.^{237,238} Conversely, other works

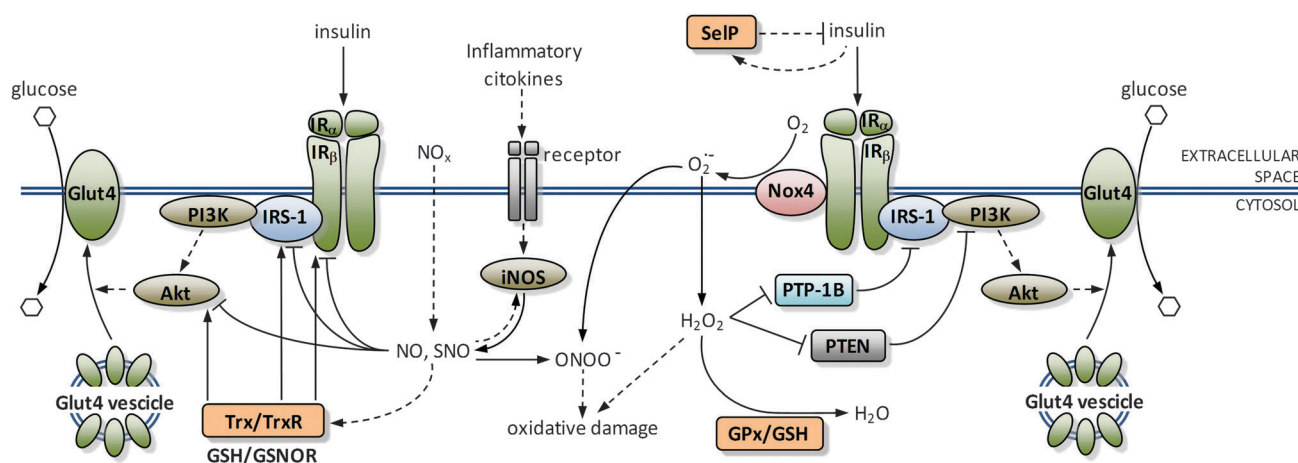


Fig. 8 Scheme of the potential role of Se-proteins in the regulation of the insulin signaling cascade.

have found high Se status in patients with diabetes, or did not observe any significant differences.^{239,240} Finally, in some cases the difference in Se status was limited to specific sub-groups such as males²⁴¹ or patients with a disease duration ≤ 2 years.²³⁴ The latest results from randomized trials confirmed such conflicting data, showing that Se supplementation could either increase the risk of type 2 diabetes or be ineffective.^{242,243} As a whole, the association between Se and type 2 diabetes appears to follow a U-shape, so that it may show variable effects depending on the position of the population baseline level of Se intake.

5.10. Endocrine disorders

Pathologic conditions directly caused by DIOs deficiency in humans have so far not been documented; however several disorders involving the metabolism of thyroid hormones are characterized by abnormal regulation of these Se-proteins. Some of these disorders have a genetic origin. A recent study identified a homozygous missense mutation of SBP2 gene as responsible for abnormal thyroid functions in humans due to decreased activity of DIO2 and unexpressed DIO1 and DIO3,²⁴⁴ a defect which cannot be corrected by SeMet supplementation.²⁴⁵ Another SBP2 gene mutation was then identified, which produces an early stop codon and results in a relatively mild clinical profile.²⁴⁶

In other endocrine disorders, altered levels of DIOs can be significantly correlated to the Se intake. A combined deficient intake of Se and iodine has been identified to be the cause of the endemic mixedematous cretinism.²⁴⁷ Several studies have linked a moderate deficiency of Se to autoimmune thyroiditis (AIT), demonstrating that long-term supplementation with SeMet or selenite entails a reduction of anti-thyroid peroxidase antibodies (anti-TPO) in most cases, with positive effects on the course of AIT.²⁴⁷ Graves' hyperthyroidism is an example of thyroid autoimmune disorder, caused by the production of autoantibodies to the thyrotropin (TSH), a receptor that stimulates the activity of DIOs, particularly DIO1, for the production of T3 and T4. Under moderate Se deficiency, Se supplementation was shown to favour the normalization of thyroid hormones metabolism, an effect that was ascribed to the increased efficacy of deiodination mediated by DIOs, coupled to enhanced contrast to the elevated level of ROS mediated by GPxs.²⁴⁸ A similar inadequate contrast to ROS by GPxs, but conversely combined with insufficient thyroid hormone synthesis due to reduced level of DIOs, may be hypothesized also in autoimmune hypothyroidism, such as Hashimoto's disorder.²⁴⁹ Therefore, a poor Se diet may be a risk factor for autoimmune thyroiditis, particularly in genetically predisposed subjects.

5.11. Male infertility

Moderate Se deficiency leads to impaired sperm motility and morphological alterations of the midpiece architecture, often resulting in disconnections of heads and tails, while in extreme Se deficiency spermatogenesis is completely abrogated.²⁵⁰ The important structural and antioxidant actions played by GPx4 in human spermatozoa make it a major candidate as a mediator of such effects. This Se-protein was recognized as one of the

possible causes of oligoasthenozoospermia, a form of infertility characterized by a reduction of both the number and the motility of spermatozoa.²⁵¹ In addition, a decrease in the expression level of GPx4 in the spermatozoa results in defected incorporation of rhodamine 123, with a loss of mitochondria membrane potential that affects their morphology.²⁵¹ As an antioxidant enzyme, GPx4 reduces phospholipid hydroperoxide and H_2O_2 , which are important secondary messengers in spermatogenesis. While these species are responsible for protamine sulfoxidation, an important process favouring sperm DNA condensation, they also contribute to oxidative stress, which may have dramatic effects on the integrity and motility of spermatozoa.²⁵² An extremely fine modulation of the messengers is necessary, in which GPx4 plays an important role. However, despite elevated level of H_2O_2 -mediated oxidative stress in spermatozoa being commonly associated with male infertility, GPx4 polymorphism or reduced activity/concentration remain unverified as causal factors in human patients.

5.12. Cancer

5.12.1. Cancer and total selenium intake/status. Selenium has become mostly known in recent years due to its assumed preventive properties against some types of cancer, mainly due to its antioxidant action. The ability of Se to reduce carcinogen induced and spontaneous cancer incidence has been widely investigated over the last 20 years in both animal and human models, in most organs, and against a broad range of cancer forms. Table 3 reports the most recent meta-analyses conducted to extrapolate a general interpretation of the relationship between Se-status and cancer risk.

Although many studies have revealed a potential association between Se status and cancers incidence, inferring a conclusive interpretation appears still to be arduous. Several studies observed an inverse correlation between Se levels and risk of prostate,²⁵⁴ bladder²⁶¹ and lung²⁵⁷ cancers. On the contrary, no significant effects were found in Se supplementation randomized trials for prostate²⁶² and colorectal²⁶³ cancers, as well as in case-control studies regarding primary liver cancer.²⁶⁴ Some indications can be gathered on the possible factors underlying the complexity of the relation between Se and cancers. Gender-dependent effects were found for colorectal cancer²⁵⁶ and a pooled set of different cancer forms,²⁶⁰ suggesting that men may respond to Se supplementation more significantly than women. Another important indication arises from the observation that Se status might show an effect on cancer risk in a limited range of levels only, whose boundaries are defined by the insufficient and saturated activity of Se-enzymes. For example, a study noticed that Se supplementation associates with a reduced risk of lung cancers in populations with baseline serum Se level $< 106 \text{ ng mL}^{-1}$, whereas increases the risk in those with Se level $> 121.6 \text{ ng mL}^{-1}$.²⁶⁵ A similar relation was observed in a meta-analysis on prostate cancer, where plasma/serum Se negatively correlated with prostate cancer risk only below 170 ng mL^{-1} .²⁵³ These findings suggest that Se exhibits a U-shape relation with cancer risk, in analogy with many other antioxidants.³ Still, all observational studies and randomized trials appear to be highly

Table 3 Selected meta-analyses on Se and of various types of cancer. RR: relative risk, OR: odds ratio; CL: confidence limits

Tissue	Number of studies	Type of study	Comparison	RR/OR (95% CL)	Ref.
Prostate	12	Randomized trial, case-control, cohort	Highest vs. lowest in plasma/serum, toenail, diet	0.29 (0.14 to 0.61) ^a	Hurst <i>et al.</i> (2012) ²⁵³
	20	Case-control, nested case-control, cohort	Pooled standardized mean difference in plasma/serum, toenail	−0.23 (−0.40 to −0.5) ^a	Brinkman <i>et al.</i> (2006) ²⁵⁴
Colorectal	7	Case-control, nested case-control, cross-sectional	Highest vs. lowest in plasma/serum	0.67 (0.55 to 0.81) ^a	Ou <i>et al.</i> (2012) ²⁵⁵
	15	Randomized trial, observational	Highest vs. lowest in blood, toenail	Women 0.97 (0.79 to 1.18) men 0.68 (0.57 to 0.82) ^a	Takata <i>et al.</i> (2011) ²⁵⁶
Lung	16	Case-control, cohort	Highest vs. lowest in serum, toenail, diet	0.74 (0.57 to 0.97) ^a	Zhuo <i>et al.</i> (2004) ²⁵⁷
Bladder	7	Case-control, nested case-control, cohort	Highest vs. lowest in serum, toenail	0.61 (0.42 to 0.87) ^a	Amaral <i>et al.</i> (2010) ²⁵⁸
Various	9	Randomized trial	Supplement vs. placebo	0.76 (0.58 to 0.99) ^a	Lee <i>et al.</i> (2011) ²⁵⁹
	7	Randomized trial	Low vs. high in serum	0.64 (0.53 to 0.78) ^a	Lee <i>et al.</i> (2011) ²⁵⁹
	4	Randomized trial	Highest vs. lowest in diet	Women 1.00 (0.89 to 1.13) men 0.77 (0.64 to 0.92) ^a	Bardia <i>et al.</i> (2008) ²⁶⁰

^a Significant.

conditioned by the composition of the population with respect to covariates and confounding factors including baseline levels of Se intake, co-supplemented antioxidants, age, gender, diet, lifestyle, time scale and others.

5.12.2. Cancer and selenoproteins. Another important factor usually unconsidered in both individual studies and meta-analyses is the actual representative parameter chosen to assess the Se status. Almost all epidemiological research has determined Se status using the total concentration of the element in plasma/serum, toenail, hair or food/supplements. Only a few studies investigated the relationship between cancer and individual serum, plasma or tissue Se-proteins concentration. A recent work reported a significantly higher level of SeAlb in early-stage colorectal cancer patients compared to the advanced stage and controls. No significant differences emerged in this case if considering GPx3, SelP or total serum Se, demonstrating that these parameters may be inadequate to figure out a complex association with cancer under non-deficient conditions. Other works did not reveal any association between colorectal cancer and total Se, GPx3 and SelP levels in plasma or serum.^{266,267} Conversely, some studies found an inverse correlation between plasma GPx3 and colorectal cancer or uterine cervix cancer,²⁶⁸ plasma SelP and various types of cancer,²⁶⁹ tissue SelP and colorectal adenoma,²⁷⁰ tissue GPx4 and pancreas²⁷¹ as well as breast²⁷² cancers. The level of TrxR1 in tumour cells was suggested to be higher than in normal tissues.²⁷³ The limited amount of epidemiological information, still contradictory in some cases, on the relationship between individual Se-species and cancer marks the importance of improving these investigations, which are often limited in sample size due to their higher analytical complexity.

More numerous are the studies in genetics and biochemical behaviour of Se-proteins in cancer tissues or cell lines. The biochemical association between Se-species and cancer is mainly mediated by their action in oxidative stress control.

Oxidative stress plays an important role in carcinogenesis by means of DNA damage induction and its effects on intracellular signal transduction pathways.²⁷⁴ Reactive oxygen species can induce almost all forms of DNA damage that have been reported in genes dysfunctions which are involved in the genesis of cancer, and play a key role in cancer development by inducing and maintaining the oncogenic phenotypes.²⁷⁵ As a consequence, genetic polymorphisms, gain or loss of functions of antioxidant enzymes, such as GPxs, has attracted great interest in the study of cancer and its therapy.²⁷⁶ The loss of the heterozygosity of GPx1 gene has been implicated in lung cancer development, while GPx1 polymorphism is associated with an increased risk of breast,²⁷⁷ bladder,²⁷⁸ hepatocellular,²⁷⁹ prostate²⁸⁰ carcinomas, and non-Hodgkin's lymphoma.²⁸¹ SelP polymorphism is associated with colorectal adenoma.²⁸² GPx3 hypermethylation has been shown to occur frequently in prostate cancer and Barrett's esophagus.⁴ Sep15 polymorphism is associated with lung cancer.²⁸³ GPx2 is upregulated in some types of cancer, particularly of gastrointestinal origin.²⁸⁴ A recent study has shown that lower expression of GPx2 increases migration and invasion of cancer cell clones, but decreases their growth, thus depending on the stage of tumour development.²⁸⁵ Finally, TrxR1 is probably the most investigated Se-protein in its relationship to cancer. This Se-protein presents both prevention and promoting properties for tumours: it regulates the redox state in the cell and activates the p53 tumor suppressor²⁸⁶ and its deficiency alters cell morphology,²⁸⁷ but it is also targeted by a number of anticancer drugs.²⁸⁸

5.12.3. Selenium in cancer therapy. A number of non-proteic Se-species have also been tested in cancer therapy for many clinical aspects. Sodium selenite systemic or topical administration elicits radioprotective effects in normal tissues.²⁸⁹ Such an effect was not observed in the corresponding malignant tissues, where dose-dependent radiosensitizing capacities, including

apoptosis induction and cytotoxicity, were on the contrary noticed.²⁹⁰ In general, the substitution of sulfur by Se in cancer chemopreventive agents is supposed to result in more effective analogues. This idea was confirmed for the action of a Se-analogue of the chemopreventive agent *S,S'*-(1,4-phenylenebis[1,2-ethanediy])bis(isothioure) (PBIT), also known to inhibit inducible nitric oxide synthase (iNOS), as an inducer of apoptosis and inhibitor of cell growth in the case of lung cancer.²⁹¹ Following the same principle, a number of organic Se-compounds have been synthesized and tested as chemopreventive agents.²⁹² The production of monomethylated Se from methylselenocysteine or methylseleninic acid has been postulated as a key step in the mechanism of Se-species anticancer activity.²⁹³ In particular, methylseleninic acid synergizes with tamoxifen to induce caspase-mediated apoptosis in breast cancer cells.²⁹⁴

An additional aspect to be considered is that Se deficiency is nearly the norm in cancer patients treated with radio- and chemotherapy, or even just hospitalized.²⁹⁵ Supplementation of cancer patients with Se at doses of up to 2000 µg per day, alone or in combination with vitamins, has been suggested as a way to improve their general quality of life.²⁹⁵

5.13. Ageing-related diseases

The relationship between Se levels and ageing is still controversial. Plasma/serum Se concentration seems to remain stable with age, but the tissue distribution may be altered.²¹³ The association of Se with ageing is generally indirect, due to the fact that most of the biological processes in which Se is involved change with age. Several studies have shown that ageing cells accumulate oxidative damage.²⁹⁶ Ageing-related oxidative stress influences many of the processes mentioned in the sections above, including damage to both mitochondrial and nuclear DNA, lymphocyte population fall, telomere length decrease in peripheral leukocytes and thyroid hormones alterations. In this context, an inadequate Se intake (even if moderately deficient) should be considered as a risk factor for many ageing-related diseases such as cancers, cardiovascular and immune disorders.²⁹⁷

5.14. Interaction with toxic metals

A particular relationship of Se with human diseases concerns its interaction with toxic metals and metalloids. Selenium has a generic antagonistic effect against metals' toxicity through a dual

action, represented in Fig. 9: direct sequestration of the toxicant and mitigation of the metal-induced oxidative stress.²⁹⁸ Low molecular weight Se-species including selenide, free SeCys and SeMet, compete with GSH, Cys and thiols in general for conjugation of the metal. Theoretically, many metal cations may form insoluble colloids or complexes with selenide, as has been observed in yeast cultures,²⁹⁹ but only silver (Ag) has been documented to accumulate in mammalian cells in this way.³⁰⁰ Conversely, Se is generally associated to a decreased bioaccumulation of arsenic (As) and cadmium (Cd), so that excretion mediated by conjugation of these elements with organic Se-species may be gathered as the favoured route.²⁹⁸ The antioxidant action of Se-proteins mitigates the cellular damage induced by metal-generated ROS. On the other hand, Se-proteins are also a target for metals' toxicity due to two effects: (i) their selenolic group make them susceptible to metal binding and consequent inactivation; (ii) each intermediate Se-metabolite engaged by the metal is also taken away from the synthesis pathway, thus from essential biological functions, resulting in a possible indirect damage.²⁹⁸ A limited number of studies investigated until now the molecular mechanisms driving these interactions, most of them focusing on As and mercury (Hg) metabolism, whereas most works were limited to observing associations between physiological/intake Se and metal levels or biological markers.²⁹⁸

The interaction of Se with As leads to a mutual inhibition of the methylation pathways and suppression of As-induced signalling, but also synergistic toxicity may be generated in some cases by the inactivation of the zinc-finger proteins.^{301,302} In animal models, arsenite and selenite were shown to react with GSH in erythrocytes and the liver, forming the complex (GS)₂AsSe⁻.³⁰² This complex is biliary excreted into the gastrointestinal tract, leading to both a counteraction of As accumulation in the organism and an alteration of Se metabolism in case of contamination. Biliary excretion of As would conversely reduce its level in the urine. A number of epidemiologic studies support the existence of an inverse association between blood Se and urinary As in humans, but the formation of Se-As conjugates has not yet been demonstrated.^{303,304}

The association between Se and inorganic Hg in exposed subjects is well established.³⁰⁵ In the bloodstream, selenite reacts also with Hg²⁺ to form a species with a core of HgSe and GS-moieties ligated on the surface in the form (HgSe)₁₀₀(GS)₅.³⁰² This species binds to SeIP up to a proportion

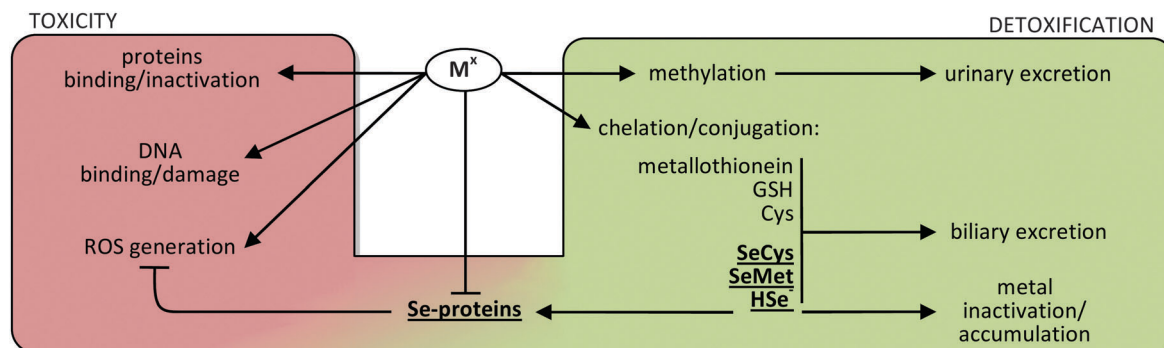


Fig. 9 Scheme of the potential role of Se-species in the toxicity and detoxification of exogenous metals (M^x).

of 35 units per protein, so that this Se-protein is actively involved in a detoxification mechanism for Hg. Methyl-Hg (MeHg) is another highly toxic species which exhibits high affinity for selenide and selenols.³⁰⁶ The potential interaction of MeHg with free SeCys, SeMet and selenide in serum (where they constitute ~10% of the Se pool) may sequester the species from thiols binding and crossing the blood-brain barrier, so limiting its neurotoxic effect. Conversely, MeHg binding to selenoproteins inhibits their activity of contrasting the oxidative stress. A number of epidemiological studies have revealed some association between supplemented seleno-species, Hg poisoning and excretion, Se-proteins activity and oxidative damage.^{307–309} However, the mechanisms appear to be difficult to extricate as low molecular weight selenospecies and selenoproteins have different roles, and Se–Hg interaction may result in antagonistic, additive, or synergistic effects depending on the context.

Among the other metals, Ag is considered an emerging pollutant of which the biochemistry and toxicity is important as much as it is obscure. The biological transformation of Ag nanoparticles has been proposed to occur by means of gastric dissolution and ions absorption, circulatory thiol transport, photoreduction to secondary Ag⁰ particles and superficial sulfidation.³⁰⁰ Reduced Se-species have also been shown to react with the surface of Ag nanoparticles. Kinetic and thermodynamic evidence support the hypothesis that Se cannot compete with the initial sulfidation, but Se/S exchange reactions occur afterwards, leading to the formation of Ag/S/Se particulate deposits in tissues.³⁰⁰ Further studies are strongly needed to shed light on the exact metabolic pathways through which individual selenospecies influence the biological transformation and toxicity of heavy metals *in vivo*.

6. Conclusions and perspectives

Remarkable progress has been achieved in recent years about the knowledge of the processes driving the biological action of Se and its species. A complex picture has emerged, where multiple Se-proteins cooperate in the regulation of transcription mechanisms, oxidative stress and redox signalling. Experiments carried out using animal models, such as knockout or overexpressing mice for specific Se-proteins, allow explicit investigation of the specific action of individual species; nevertheless uncertainty rises over the reliability of results when extended to humans under normal conditions. The lack of a clear definition of what is a “normal” Se status, and a corresponding adequate set of parameters to assess it, prevents the identification of those factors related to health disorders.

The complexity of this issue lies in a number of interacting key aspects, as follows: (i) bioactive Se-species intervene on the equilibrium of biological functions in a quantitative way. As well being represented by the example of immune response, Se-proteins may play opposite actions with other substances or themselves, depending on the biochemical context, the regulatory mechanisms and the relative concentration/activity. A wide

variety of new methods have been developed in recent years for the quantification of Se-species, among which Se-proteins are the most challenging.³¹⁰ A full integration of these methods with qualitative biological and clinical approaches will provide new tools to elucidate the unknowns in the Se-regulated processes. (ii) Genetic polymorphism of Se-proteins has arisen as a potential forcing variable in the regulation of both Se status and disease risk, progression and prognosis. Exhaustive studies are strongly needed to elucidate how genetic factors influence the response of the organism to the Se intake and metabolism under disease conditions. (iii) The advancement in the understanding of genetic and biochemical processes must be used to drive epidemiological studies in detail. Despite the large number of both observational studies and randomized trials that have been conducted over the years, many of them have been criticized because of the inadequate selection criteria or scarce collection of complementary data. Particular attention should be paid to the genetic characterization of subjects, the distribution of Se status within the population, and the quality of the marker(s) adopted for assessment of Se status.³¹¹ Finally, a number of unresolved questions need to be dealt with, such as the dynamics and regulation of the biosynthesis of Se-proteins, and the comprehension of the biochemical function for most of the Se-proteins.

The emergent discipline of Systems Biology offers promising tools to integrate all these key aspects, by combining large amounts of experimental data coming from genomics, transcriptomics, proteomics and metabolomics, to generate comprehensive networks models.³¹² A number of Bioinformatic applications like *Gene Ontology* (GO) or *List2Networks* can be used to integrate experimental data with existing databases, to generate protein–protein interaction and gene regulatory networks. Network analysis could allow the detection of potential key features of the complex Se-proteins system, such as: the existence of gateway proteins (hubs) in biochemical pathways; new regulatory mechanisms of the global and local Se status; reactions and robustness of the system to be perturbed due to the altered intake, disease onset/progression and pharmacological treatment. Overall this new information may constitute an important base to figure out the dynamics of Se-proteins-regulated processes under normal status situations, to predict the changes under altered diet and health conditions, and to drive reliable epidemiological studies.

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